BULLETIN OF EXPERIMENTAL BIOLOGY
& MEDICINE

VOLUME 43 NUMBER 4 1957

PHYSIOLOGY

IONO-HUMORAL INTERRELATIONS IN THE ORIGIN AND SPREAD OF VAGAL INHIBITION OF THE HEART

Article II

THE EFFECT OF ACETYLCHOLINE AND K-IONS ON THE NORMAL AND ATROPINIZED FROG'S HEART

I. A. Keder-Stepanova

From the Laboratory of Electrophysics, S. P. Botkin Clinical Hospital, Moscow

(Received December 29, 1954. Presented by M. S. Vovsi, Active Member of the Academy of Medical Sciences, USSR)

During the development of vagal inhibition of the heart, the tissues secrete acetylcholine [19], and the K-ion concentration in the perfusate is increased [6, 11, 15, 18, 23]. For this reason, physiologists believe that the development of a vagal effect is determined by the ultimate effect of either acetylcholine or K-ions on the cardiac muscle.

Both seem probable, since the acetylcholine on the heart is attended by K-ion secretion (Lehnartz, [17] – turtle heart; Holland, [14] - guinea pig auricles), and acetylcholine appears when the concentration of K-ions increases in a heart perfusate (Beznak [7]). Physiologists base the association of vagal inhibitory development with the ultimate effect of K-ions or acetylcholine on the phenomenological similarity of the effects caused by vagus nerve stimulation and by the action of these substances extraneously introduced. This is probably the reason why there are so many works studying the "inhibitory" effect of acetylcholine and K-ions.

Actually, such an important sign of vagal inhibition as a decreased cardiac contraction amplitude can be observed during the action of both K-ions and acetylcholine. Many authors mention the slowed rhythm and subsequent cessation of the heart under the influence of these agents. The problem of the polarized shifts which are observed in the heart with the action of acetylcholine or of K-ions is widely discussed.

Using a needle and mirror galvanometer, A. S. Mozzhukin [4], V. B. Boldyrev [1], and V. B. Boldyrev and P. A. Kiselev [2] observed the resting current of the cardiac muscle to increase under the influence of acetylcholine.

V. B. Boldyrev [1] recorded an increase in the resting current during the action of surplus K-ions.

However, there are contradictory data. It has been definitely established that K-ions actively depolarize different stimulated tissues [9, 10, 12]. There are indications that acetylcholine cannot increase the polarization of the cardiac muscular structures [3, 5, 21, 22].

We propose that neither acetylcholine nor K-ions alone can produce those changes in the functional properties of the cardiac muscle which are observed during the development of vagal inhibition. Only a mutually caused disturbance of the ionic balance, the release of acetylcholine and the change in the polarization faculty of the cardiac muscle cellular structures can bring about the initiation and development of the complex process of cardiac inhibition.

The purpose of this work was to study the action of acetylcholine and K-ions on a frog's heart.

EXPERIMENTAL METHODS

The experiments were done on the hearts of male and female Rana temporaria over a two-year period from 1947-1949. We used the rotary perfusion method and also a preparation made from a heart isolated according to Shtraub; heart action was electrocardiographically recorded in strictly potentiometric conditions on a string galvanometer connected with a continuous current booster by a direct connection (for details of methods, see Article I). Acetylcholine was prepared in a Ringer's solution from a dry preparation of acetylcholine hydrochloride. The concentration of the potassium solution was usually 10^{-3} (1 g of potassium chlorate to 1,000 ml of Ringer's solution). Therefore, the K-ion content in the active solution was 0.114% instead of the 0.014% in an ordinary Ringer's solution.

Two series of experiments were done.

EXPERIMENTAL RESULTS

The first series of experiments studied the action of acetylcholine and of potassium on a normal frog's heart. About 200 tests were done to determine the effect of acetylcholine in concentrations of 10^{-5} to 10^{-9} on the isolated frog's heart.

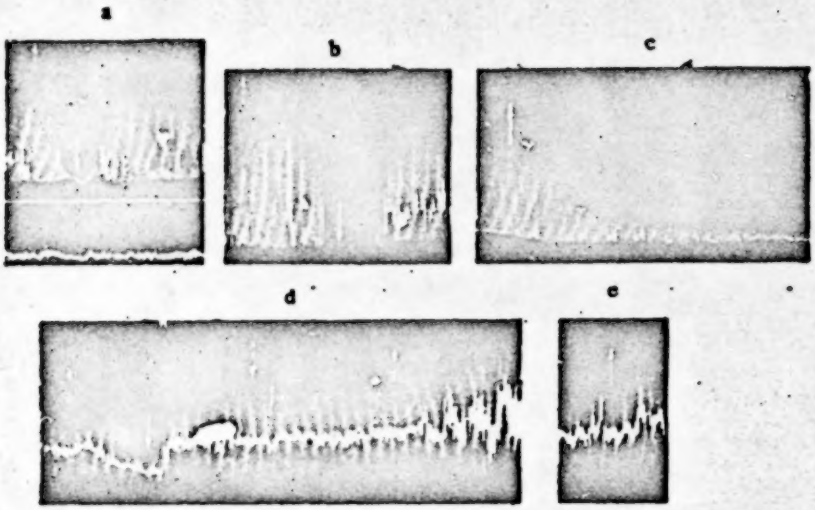

Fig. 1. The action of acetylcholine on an isolated frog's heart in conditions of Shtraub's method (a,e) and of rotary perfusion (b,c,d): a, b, c) reaction of a normal heart; d) reaction of normal and atropinized heart (atropine 10^{-5}); e) reaction of a previously desympathized and atropinized heart. Acetylcholine concentration: a, d, e) 10^{-7}; b) 10^{-6} c) 10^{-8}.*

A negative inotropic effect, which depended on the concentration of the substance administered, was observed in all cases. Figure 1, a,b, and c show the effect of acetylcholine in concentrations of 10^{-7}, 10^{-6} and 10^{-8} respectively. Kymogram a, in Fig. 1, illustrates an acetylcholine injection into a heart isolated according to Shtraub. Figure 1, b and c show the action of acetylcholine when it was administered directly to the sinus venosus by rotary perfusion. In no case were any changes in the inherent rhythm of the heart recorded. The apparent retardation of rhythm in Fig. 1, a and b is the result of blocking atrioventricular conduction 1:2 and

* ...o explanation for arrows given in Russian text – Publisher's note.

1:4 (in these cases, the electrocardiogram showed a normal sinus rhythm). In Fig. 2, one can see the effect of acetylcholine on the form and duration of development of the action potentials, and also on the resting potential level of the cardiac muscle; mechanograms of the auricles and ventricles were recorded simultaneously with the electrocardiogram. Acetylcholine, especially, sharply changed the monophasic action potential tracing, destroying its normal plateau and accelerating the development of the descending limb, i. e., the restorative processes after depolarization.

Reports have recently appeared in the literature which agree with the data we obtained; Hoffman and Suckling, especially, observed while recording monophasic action potentials of a dog's heart with intracellular electrodes that the repolarization processes were accelerated under the influence of acetylcholine.

However, the acceleration of the restorative processes was attended by the development of residual negativity; the dssue could not fully restore the original potential, and the resting potential was observed to decrease (an upward shift of the base line, which occurred as a secondary phenomenon after the development period of the monophasic action potentials had been reduced – see Fig. 2, a).

In cases where an atrioventricular blockade developed and where there were no ventricular monophasic potentials, the sinus rhythm was unchanged (Fig. 2 b), and a small secondary increase in the resting potential was observed.

Therefore, the injection of acetylcholine into the heart (either into the ventricles or directly into the sinus venosus) was attended by a decrease in contraction amplitude, the degree of which depended on the concentration of the acting substance. High concentrations of acetylcholine caused a blockade of the atrioventricular and sino-atrial conductions to develop. The fact that acetylcholine did not affect the level of the resting potential of the cardiac muscle directly must be emphasized. This could only be detected when working with a potentiometric apparatus by which one could record the primary effect of acetylcholine on the restorative processes (repolarization processes).

The effect of surplus K-ions on an isolated frog's heart was tested 120 times. In no case did the recordings show the inherent rhythm of the heart to be retarded. In 40% of the experiments, a positive chronotropic effect was observed, reaching 10% (rhythm accelerated to 6-7 contractions per minute). As a rule, the action of K-ions in a 10^{-3} concentration was attended by a considerable negative inotropic effect, sometimes involving the disappearance of the ventricular contractions. The kymogram in Fig. 3,a was obtained from K-ions acting (in a concentration of 10^{-3}) directly on the ventricles, and the one in Fig. 3,b from the same action on the sinus venosus of a frog's heart. In Fig. 3,a, one can see the effect frequently caused by the action of potassium – a blockade of the atrioventricular conduction developing. Sometimes a sino-atrial or even a sino-sinusal blockade was observed.

Studying the effect of K-ions on the bioelectric tonicity of the cardiac muscle was especially interesting. The experiments were conducted in the usual way. A typical result of the K-ion action is shown in Fig. 2, d. The injection of potassium in a 10^{-3} concentration was attended by a sharp decrease in the resting potential (i.e., negativation) of the cardiac muscle, with a simultaneous decrease in the amplitude and development time of the monophasic action potential. We observed a negative inotropic and a positive chronotropic effect on the mechanogram of this experiment.

In many cases, a sharp alteration of the monophasic action potential was observed, which undoubtedly indicates that the depolarization processes are disturbed by K-ion action.

In the case of the cardiac muscle also, therefore, K-ions are a factor actively depolarizing the cellular structures of the myocardium.

The second series of experiments examined the action of acetylcholine and K-ions on an atropinized frog's heart.

In 1924, O. Loewi and V. Navratil [20] concluded that acetylcholine and atropine in the cardiac muscle were interrelated. The authors believed that atropine reduced the sensitivity of the cardiac muscle to additionally administered acetylcholine or to that secreted by the vagus nerve endings. In spite of the many works treating this problem, there is still no clear understanding of the changes effected by the action of acetylcholine on an atropinized heart.

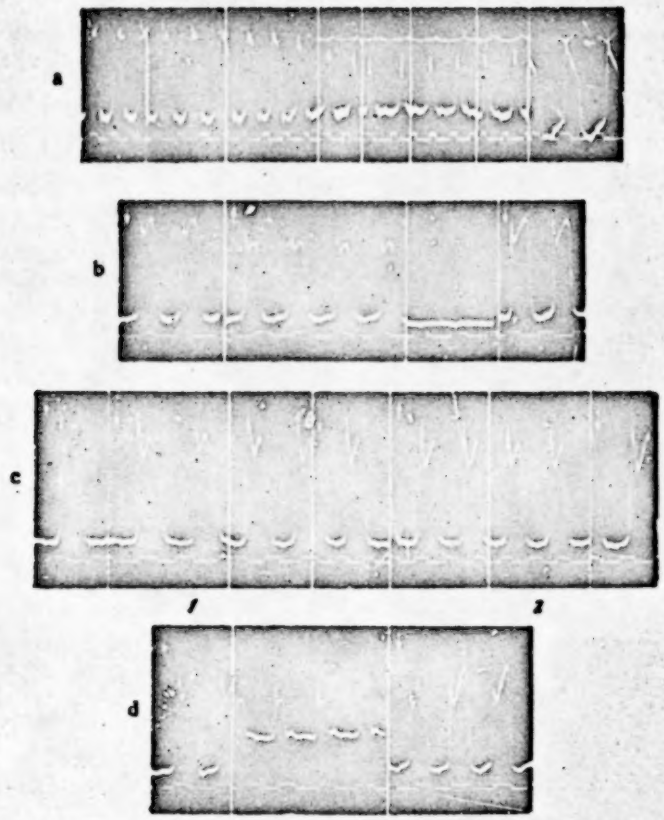

Fig. 2. Change in monophasic action potentials and resting potential of an isolated frog's heart during the action of acetylcholine in a 10^{-7} concentration (a,b,c) and of K-ions in a 10^{-3} concentration (d). The tracing b shows the effect of acetylcholine on a previously atropinized heart. Explanation of tracings: from top to bottom: a) mechanogram, electrogram, time indication; b, c, d) electrogram, time indication. Arrows mean the same as in Fig. 1.

The effect of acetylcholine in different concentrations on a previously atropinized frog's heart was tested 270 times. After the effect of acetylcholine in a Ringer's solution had been recorded, atropine was introduced into a cannula (usually in a concentration of 10^{-5}), and, after a fixed time interval, the effect caused by the same solution of acetylcholine was examined. After the introduction of acetylcholine into the atropinized heart, a positive inotropic effect developed in 256 cases.

Immediately after the Ringer's solution was replaced with the atropine, the usual negative inotropic result effected by acetylcholine was observed to decrease. One to two minutes after the administration of atropine, acetylcholine had no effect on the force of the heart contractions. However, after 2-5 minutes, a second test showed a positive inotropic effect from the action of acetylcholine in the same concentration. The supposed reduced sensitivity of the atropinized heart to acetylcholine should have been evidenced by different reactions to different concentrations of acetylcholine, i. e., in the course of atropinization, a heart which did not react to a 10^{-7} concentration of acetylcholine should have reacted to a 10^{-6} or 10^{-5} concentration, even if only by a weak negative inotropic effect. We did not observe in any case such a gradation in atropinized heart reactions.

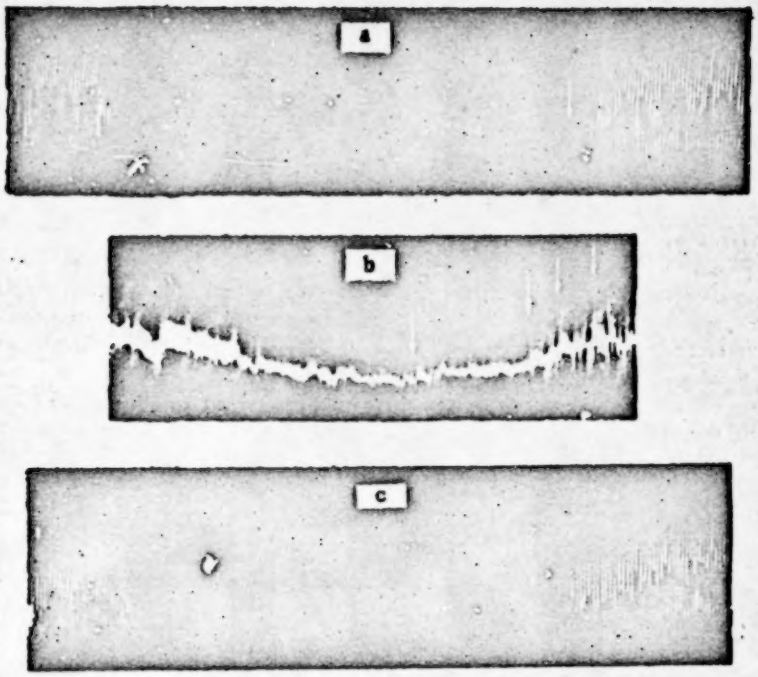

Fig. 3. Effect of surplus K-ions on frog's heart isolated according to Shtraub (a) and under conditions of rotary perfusion (b); c) effect of K-ions on a previously atropinized heart (atropine in a 10^{-5} concentration). The arrows mean the same as in Fig. 1.

Any concentration produced the sequence described above in the development of a positive inotropic effect. There was no change in the inherent rhythm of the heart in any case.

Figure 1, d shows the positive inotropic effect which occurred when acetylcholine was administered in a concentration of 10^{-7} after the 2-minute action of atropine.

Many authors, having observed a positive inotropic effect from acetylcholine under conditions of atropinization, propose that it develops from the action of acetylcholine on the endings of the cardiac muscle sympathetic fibers.

The experiments we conducted on previously desympathized hearts showed that, in 44 out of 52 cases, a clearly expressed, positive inotropic effect was observed from acetylcholine in any concentration (see Fig. 1,e).

Then we studied the action of acetylcholine on the bioelectric tonicity of an atropinized heart. Identical results were obtained in all 56 tests. The electrogram in Fig. 2, c shows that the atropine administration caused the resting potential to slightly increase (downward shift of the base line). The action of acetylcholine in a 10^{-7} concentration on an atropinized heart was attended by a decrease in the resting potential (tracing II — upward shift of base line); in this case, the mechanogram showed a positive inotropic effect.

Therefore, when the heart is atropinized, acetylcholine can no longer accelerate the restorative processes (repolarization) which occur after depolarization; it only lowers the resting potential of the atropinized muscle to the normal level.

In 1936, Lehnartz [17] established that the heart, after atropinization, cannot release K-ions in response to vagus nerve stimulation and to the action of acetylcholine. This fact is extremely important in determining the

role of loco-humoral interrelations in the development of vagal inhibition of the heart. In connection with this, we studied the action of K-ions on a mechanogram and electrogram of the atropinized heart.

When Fig. 3, a is compared with Fig. 3,c, one will observe that there is no difference in the reactions of normal and atropinized hearts to the action of surplus K-ions in a 10^{-3} concentration. Analogous results were obtained in the electrogram recording of the heart; the negativation of the atropinized cardiac muscle was the same as under normal conditions.

There are many works in the literature studying the effects of acetylcholine and K-ions on the normal and atropinized heart. All these works start with a comparison and description of the "inhibitory" effect of acetylcholine, K-ions and vagus nerve stimulation. From this, the conclusion seems logical that "atropinization of the heart, which relieves vagal and acetylcholine inhibition, does not prevent the development of inhibition due to the influence of potassium" [1]. The same author speaks of "the extremely fine differentiation of acetylcholine and potassium inhibitory mechanisms" when the heart is atropinized. However, it seems to us that there is no basis for these conclusions. Vagal inhibition is attended by an increase in the polarization of the cardiac muscle's cellular structures and by the cessation of activity in the sino-atrial node. No similar occurrences were observed under conditions of acetylcholine or K-ion action. Acetylcholine and potassium decrease the force of the heart contractions and aid the development of conduction blockade. Acetylcholine, which accelerates the restoration of the initial potential, decreases its level, i. e., indirectly lowers the polarization of the cardiac muscle. Potassium actively depolarizes the cellular structures of the myocardium, but does not affect the original rate of rhythm. We suggest that neither of these agents are inhibitory, i.e., neither can cause all the signs of vagal inhibition of the heart. Therefore, it is difficult to propose that atropine can "differentiate acetylcholine and potassium inhibition mechanisms".

When the action of acetylcholine on a normal heart is compared with that on an atropinized heart, it appears that atropine does not eliminate the effect of this substance, but only changes the reaction of the heart to the acetylcholine. Atropine, which stabilizes the original polarization of the cellular structures of the heart (see Article II), prevents the acceleration of repolarization under the influence of acetylcholine.

When Lehnartz's data is considered, one can suppose that, under conditions of atropinization, the correlation of the different ions in the cardiac muscle is disturbed, and vagus nerve stimulation is not attended by the emission of K-ions from the tissues. We observed an intensification of the positive effects from acetylcholine on atropinized hearts of spring and fall frogs. In the spring and fall, a positive inotropic effect could be obtained, even on a normal heart, from the action of acetylcholine. In these periods, the correlation of the K-ions and calcium in the tissues is known to change greatly [8, 16, 24].

All these facts only prove the necessity of normal ionic relations to the realization of the usual effect of acetylcholine, but do not solve the problem of how this substance or K-ions "inhibit".

However, we hope that the data we have obtained will help approach an understanding of how the ionic, humoral and polarizing factors are interrelated in the origin and spread of vagal inhibition of the heart.

SUMMARY

The effect of acetylcholine and K-ions upon the rate and force as well as upon the resting and action potentials of normal and atropinized frogs' hearts was studied (over 600 experiments). Acetylcholine and K-ions diminished the force of contraction of the normal heart and promoted the blocking of conductivity, the rate of sinus rhythm being unaltered. Acetylcholine accelerated repolarization leading to a somewhat diminished polarization of the heart muscle. Potassium suppressed the contractions of the heart muscle diminishing at the same time the amplitude and slowing the development of monophasic action potentials. Preliminary atropinization did not alter the effect of surplus potassium on the heart. Acetylcholine however did not accelerate repolarization under these conditions, it only lowered the resting potential of the atropinized muscle down to its normal level. Acetylcholine enhanced the force of contraction of the atropinized normal and permanently desympathized heart.

LITERATURE CITED

[1] V. B. Boldyrev, in the book: Works of the Leningrad Medical Institute of Sanitary Hygiene," 1950, part 7, pp. 199-215.

" In Russian.

[2] V. D. Boldyrev and P. A. Kiselev, Works of the Leningrad Medical Institute of Sanitary Hygiene,* 1956, part 7, pp. 216-222.

[3] I. A. Keder-Stepanova and M. G. Udelnov, Fiziol. Zhur. SSSR, 1951, Vol. 37, pp. 180-185.

[4] A. S. Mozzhukin, in the book: Collection of Works Dedicated to the 65th Birthday of Academician L. A. Orbeli,* Leningrad, 1948, pp. 233-244.

[5] M. G. Udelnov, in the book: Summaries of the Proceedings of the VIII All-Union Conference of Physiologists,* Moscow 1947, pp. 620-621.

[6] L. Asher, Ztschr. f. Biol., 1923, Bd. 78, S. 297-314.

[7] A. B. Beznak, J. Physiol., 1934, Vol. 82, pp. 129-153.

[8] N. Brock, Arch. f. exp. Path. u. Pharm., 1941, Bd. 198, S. 609-643.

[9] K. Cole, in the book: Cold Spring Harbor Symposia on Quantitative Biology, 1940, Vol. 8, pp. 110-122.

[10] K. Cole and H. J. Curtis, J. Gen. Physiol., 1941, v. 24, pp. 551-563.

[11] N. Gavrilescu and N. Jonescu, Compt. rend. soc. biol., Vol. 124, pp. 971-972.

[12] A. L. Hodgkin and A. F. Huxley, J. Physiol., 1945, v. 104, pp. 176-195.

[13] B. F. Hoffman and E. E. Suckling, Am. J. Physiol., 1953, v. 173, pp. 312-320.

[14] W. C. Holland, C. E. Dunn and M. E. Greig, Am. J. Physiol., 1952, v. 168, p. 546.

[15] W. Howell and W. Duke, Am. J. Physiol., 1908, v. 21, pp. 51-63.

[16] B. Kisch, Arch. f. exp. Path. u. Pharm., 1929, Bd. 116, S. 227-233.

[17] E. Lehnartz, J. Physiol., 1936, 86, pp. 37-38.

[18] E. G. Lipow, S. J. Reed, and W. K. Weaver, Proc. Soc. Exp. Biol. a. Med. 1931, 28, pp. 531-533.

[19] O. Loewi, Arch. ges. Physiol., 1921, Bd. 189, S. 239-242.

[20] O. Loewi and E. Navratil, Arch. ges. Physiol., 1924, Bd. 206, S. 123-134.

[21] W. Rössel, Arch. f. ges. Physiol., Bd. 250, S. 200-227.

[22] K. E. Rotschuch, Arch. f. ges. Physiol., 1952, Bd. 255, S. 367-378.

[23] N. Scheinfinkel, Ztschr. f. Biol., 1925, Bd. 82, S. 285-290.

[24] K. Wachholder and F. Nothmann, Arch. f. ges. Physiol., 1932, Bd. 229, S. 120-132.

* In Russian.

CONDITIONED RESPIRATORY REFLEXES IN CATS

L. A. Toporkova

From the Chair of Normal Physiology, Kuibishev Medical Institute (Head – Prof. M. V. Sergievsky, Corresponding Member of the Academy of Medical Sciences, USSR)

(Received January 16, 1957. Presented by Prof. V. V. Parin, Active Member of The Academy of Medical Sciences, USSR)

The purpose of this work was to study, by the conditioned reflex method, the features of grey matter respiratory regulation and the functional links of the respiratory center with various analysor regions of the cat's cerebral cortex.

There are two works known to us in the literature which study conditioned respiratory reflexes in cats. A. A. Markova [2] produced conditioned respiratory reflexes and differentiations to a metronome in male cats before and after castration. N. Yu. Belenkov, G. Potoreiko and R. Startseva [1] established the possibility of forming conditioned respiratory and motor reflexes to light and sound stimuli in cats from which the cerebral cortex had been removed.

EXPERIMENTAL METHODS

A total of 186 experiments were done on 4 animals (3 males and 1 female). In all of the animals, effective conditioned respiratory reflexes of short duration were formed to the light of a 40 W bulb and a bell (frequency, 3 times per second). The unconditioned, reinforcement stimulus was ammonia fumes (10-15%), which were blown from a phial through a rubber tube and funnel into the animal's nose.

The isolated action of the conditioned stimulus was continued for 5-10 seconds, and then for 5-10 seconds simultaneously with the unconditioned. In each experiment, a 5-8 combination was used with intervals of 1-5 minutes. Differentiations were produced to a "red light" bulb of 40 W and to a bell rung once per second. The respiration was recorded by pneumograph.

EXPERIMENTAL RESULTS

In all of the experimental animals, the unconditioned stimulus (ammonia) caused a clear and continuous weakening of the respiratory movements. The breathing became more rapid as well as weaker in the male cat Bars and in the female Mura.

We began the main experiments after completely eliminating the tentative motor and respiratory reactions to the conditioned stimuli.

The experimental data given in the table shows the definite differences in the behavior of the animals and in the conditioned respiratory reflex and differentiation formation dynamics, which is associated with the individual features of the highest nervous activity in the various experimental animals.

The rapid formation and fixation of the conditioned reflexes and differentiations in the male cats Pestry and Bars indicate a comparatively high force and mobility of the nervous processes. The slow, gradual formation and fixation of the conditioned reflexes and differentiations in the male cat Skripun is evidently connected with a certain inertia in the nervous processes.

In the female (weak and unstable), in contrast to the males, the conditioned respiratory reflexes and differentiations were more slowly produced and remained weakly expressed and unstable throughout the experiment.

In all of the animals, the conditioned respiratory reflex mirrored the unconditioned in the change of the amplitude and frequency of the respiratory movements. In the male, Bars, and the female, Mura, it was expressed by weakened and, at the same time, more rapid respiratory movements. See the pneumogram of the male cat Bars (Fig. 1) for illustration.

In the male cats Pestry and Skripun, the conditioned reflex was expressed by a sharp weakening of respiratory movements, but there was no change in respiration rate.

The degree to which the conditioned respiratory reflexes were expressed increased according to the extent to which they were fixed, and the latent period was reduced by 0.5-1 second.

Therefore, in contrast to A. A. Markova's data, the conditioned reflex changed the depth and rate of the respiratory movements simultaneously in two of the animals in our experiments. In the first experiments, foreign and unusual stimuli (movement of objects in the field of vision, teeth gnashing, noise, coughing) caused inhibition of the conditioned respiratory reflexes according to the type of exterior inhibition. In the later experiments, according to the degree to which the conditioned respiratory reflexes were established, these same stimuli did not inhibit but rather intensified the degree to which the conditioned reflexes were expressed in the cats. On this basis, one can suggest that the stimulation threshold, arising in the cerebral cortex to the conditioned stimulus, becomes dominant according to the extent to which the conditioned respiratory reflex is fixed

Formation of Conditioned Respiratory Reflexes and Differentiations

Name and brief description of animal	Conditioned respiratory reflexes		Differentiations	
	to bell	to light	to bell	to light
Pestry – young male, playful and active. Weight 2,700 g	After 2nd-3rd combination; became permanent after 7th-8th combination	After the 9th-10th combination; became permanent after the 13th-14 combination	Complete and permanent after the first use	After the 14th use; became permanent and complete gradually
Bars – young male, pugnacious, active, greedy. Weight 2,600 g	After the 3rd combination; became permanent after the 7th-8th combination	After the 5th combination; permanent	Complete and permanent after the 4th-5th use	Complete and permanent after the 16th use
Skripun – male, rather inactive, agressive. Often fell asleep during the experiment; Weight 3,500 g	After the 6th combination; became permanent after the 15th-16th combination	After the 9th-10th combination; did not become fixed for a long time (until the 30th-35th combination	Incomplete from the first use to the end of the experiments	75 W bulb. After the 10th use; incomplete throughout the experiments
Mura – young female, rather inactive, timid. Took a long time to become accustomed to the cage. Weight 1,800 g	After the 8th combination; unstable and weakly expressed throughout the experiments	After the 14th-15th combination; unstable and weakly expressed	Not produced	After the 17th-18th use; incomplete and unstable

The conditioned respiratory reflexes were rather permanent. The use of the conditioned stimulus without reinforcement several times during the experiment did not remove the conditioned respiratory reflexes, nor did interruptions of 2-3 days to one month in the experiments.

After the conditioned respiratory reflexes had been fixed, differentiations were produced to stimuli similar to the conditioned ones. In the male cats, the differentiations formed rather quickly – to the bell in the very

Fig. 1. Pneumogram of respiration in the male cat Bars. Conditioned respiratory reflex during the 7th and 8th combination of the stimuli. Key to tracings (from top to bottom): pneumogram; indication of conditioned stimulus (bell); indication of conditioned stimulus plus unconditioned (ammonia); indication of time (in 1 second marks).

first experiment at the 1st-4th use and a little later to the light of the red bulb; in the 2nd-3rd experiment after the 10th-16th use of the conditioned stimulus alone, without the reinforcement.

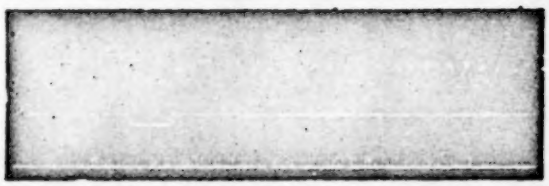

Fig. 2. Pneumogram of respiration in the male cat Pestry. Tracings the same as in Fig. 1. 1) Conditioned respiratory reflex during the action of a positive stimulus (white light); 2) absolute differentiation to the light of a red bulb. Tracings mean the same as in Fig. 1.

The differentiations rapidly acquired absolute meaning in the male cats Pestry and Bars. For example, the pneumogram of the male cat Pestry (Fig. 2) shows a marked weakening of respiration during the action of the positive stimulus (1 – white light of a 40 W bulb), but no change in respiration after the 27th use of the differentiation stimulus (2 – red light).

The formation of absolute differentiations to a red light is probably not caused so much by the difference in color as by the difference in the intensity of the light.

A different differentiation was produced in the male cat Skripun than in the other experimental animals – a differentiation to the light of a 75 W bulb, but this differentiation never acquired absolute meaning throughout the experiments.

The differentiation stimuli first (2-3 seconds) caused a slight weakening in the respiration, which then returned to normal during the stimulation period. The phenomenon of successive inhibition was observed in two of the males, Pestry and, especially, Bars.

The use of the positive stimulus 10-30 seconds after the differentiation stimulus did not cause a conditioned respiratory reflex. See the pneumogram of the male cat Bars for illustration (Fig. 3).

The respiration became uneven in depth and frequency in all of the experimental animals during the mating period (period of sexual excitation).

Both the positive and inhibitory conditioned stimuli often caused the animal to give a strong motor reaction and to cry out. The conditioned respiratory reflexes and the differentiations were disturbed, and the positive reaction to the bell remained only in the male cats Pestry and Bars. The biologically more important, dominant sexual reflex removed the temporary links between the respiration center and the cortex of the cerebral hemispheres and destroyed the normal interrelation between the processes of stimulation and inhibition. After the mating period, even breathing was restored, and the conditioned respiratory reflexes and differentiations returned as before.

Fig. 3. Pneumogram of respiration in the male cat Bars. 1) Conditioned respiratory reflex during the action of a positive conditioned stimulus (40 W white light); 2) absolute differentiation to conditioned stimulus (40 W red light); 3) inhibition of the conditioned reflex to a positive stimulus used 20 seconds after the differentiation stimulus (successive inhibition). The tracings mean the same as in Fig. 1.

Works conducted in the I. P. Pavlov laboratories indicate that conditioned reflexes (motor, secretion) in dogs are more quickly formed to sound stimuli than to light stimuli.

This fact proved to be true in our experiments also, as the conditioned respiratory reflexes and differentiations to the sound stimulus were formed and became permanent and stable more rapidly in all of the experimental animals than those to the light stimuli.

On this basis, one can propose that the functional links of the respiratory center (both positive and negative) with the auditory analysor are more stable and permanent in cats than the functional links with the visual analysor. We make this proposal on the basis of the theory developed by M. V. Sergievsky [3] that "the different regions of the cortex are not equal in respect to respiratory regulation in the higher mammals and in man; The respiratory center of the medulla oblongata has more stable links with some regions of the cortex than with others. These stabler links are determined by the whole character of the analysor and synthetic activity of the given cortical regions*.

SUMMARY

Four cats (3 male) were under investigation. Conditioned respiratory reflexes and differentiation to light (40 w lamp) and sound (a bell of various frequencies) stimuli were established and reinforced by ammonia (unconditioned stimulus).

A rate and stability of established conditioned respiratory reflexes and differentiation differed and depended on the individual properties of the animal's higher nervous activity.

The quality of conditioned respiratory reflexes was the same as the unconditioned and when firmly established acquired dominant properties. All animals established conditioned respiratory reflexes and differentiation more readily to sound than to light stimuli.

* In Russian.

In two animals phenomena of successive inhibition were noted. During the mating period conditioned respiratory reflexes and differentiation disappeared, but were subsequently restored.

LITERATURE CITED

[1] N. Yu. Belenkov, G. Potoreiko and R. Startseva, Byull. Eksp. Biol. i Med., 1956, Vol. 41, No. 2, pp. 17-20.

[2] A. A. Markova, in the book: Questions of the Comparative Physiology and Pathology of Highest Nervous Activity," Leningrad, 1955, pp. 69-78.

[3] M. V. Sergievsky, Respiratory Regulation by the Cerebral Cortex," Moscow, 1955.

ON THE RECIPROCAL ACTION OF THE NERVE CENTERS OF THE MOTOR ANALYSOR

E. A. Mukhamedova

From the Department of Physiology, Central Institute for Physical Culture Research, (Director Assistant Prof. N. G. Ozolin), Moscow

(Received August 29, 1956. Presented by Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

In sports, athletes often tense muscle groups which do not directly affect the realization of a movement – tension in the arm, face and neck muscles of a finishing runner, constriction of a skater's arms, joined behind his back, when his foot is jarred, change of expression and movement of the head in a tired athlete, etc. Evidently, the inclusion of such "supplementary" muscular groups in the action is one of the factors increasing the efficiency of muscular activity.

The reciprocal action of the nerve centers causing the realization of coordinated movement was first observed by N. E. Vvedensky [2] in a study of the interrelations between the musculatures of animals' legs. N. E. Vvedensky and A. A. Ukhtomsky [3, 4] showed that the activity of muscle antagonists is not always opposed but can also be synergistic. The authors point out that the reciprocity of reactions is determined, not beforehand by a predetermined antagonism of the centers, but by the degree of activity in one of the centers, thereby promoting a new functional dynamics principle of reciprocity.

There is cross-innervation in human arms, similar to the cross-coordination found in animals' legs [11, 12], and these functions can also be synergistic [1, 15].

Such a phenomenon of opposite reactions is evidently explained by the different conditions present in the experiments of the various authors, which promoted various types of reaction.

In 1903, I. M. Sechenov [14] showed that efficiency can be more quickly restored to a fatigued right arm when the left arm is in action. Sechenov explained this phenomenon by the fact that "the source of fatigue is not in the muscles, but in the processes occurring in the nerve centers".

The works of later authors, who studied work and active rest conditions in more detail, [6, 7, 8, 9, 10, 13, 16] show that the effect of a muscular contraction can change depending on the conditions of activity present in other muscular groups.

The purpose of our work was to study the effect of contractions in "supplementary" muscle groups on the efficiency of muscular activity.

EXPERIMENTAL METHODS AND RESULTS

We conducted 5 series of experiments studying the force, speed, duration and frequency of movements in different kinds of muscular activity. The results of several variants of the same simple movement were examined in each experiment.

In the first series of experiments, the maximal muscular tension in "sitting" and "at ease" positions was determined in 41 men by measurement with a hand dynamometer. The tension of muscular groups which did not directly participate in pressing the dynamometer in the "sitting" position, namely, the muscles of the trunk

and legs, was excluded. In the "at ease" position, the subject pressed the dynamometer in the pose most comfortable to him. The majority of the subjects, in this case, performed a series of movements – the arm was abruptly dropped down from the dynamometer, the subject squatted and inclined the trunk forward. Then the other arm usually became tense and the jaws clenched.

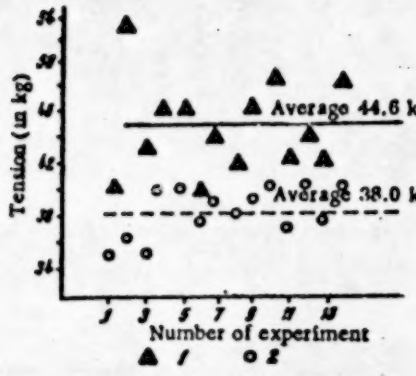

Fig. 1. Hand dynamometry in the "sitting" and "at ease" positions.
1) "at ease"; 2) "sitting"

In 95 out of 100 cases, greater muscular force was shown in the "at ease" position, in 4 cases, the results were identical in both positions, and in only one case, greater muscular force was shown in the "sitting" position. The increased force in the "at ease" position was, on the average, 12% as compared with the "sitting" position; individual fluctuations reached 30-40%.

Figure 1 shows the change in the hand dynamometer in one of the subjects.

The force of muscular contraction was studied with an inertia dynamometer in the second series of experiments. The principle of the inertia dynamometer, which was constructed by N. N. Goncharov [5] consists in contrasting the force of inertia with the force of muscular contraction. Flexing the arm at the elbow in the frontal plane with maximal force, the subject stretched the rope, unwinding the discs. According to the speed at which the discs rotated, which was automatically recorded, the force, rate, time and their derivatives – the work and power of the forearm flexion and also the change in these indices during one flexion – could be determined.

When the right forearm was flexed, the force of the muscular contraction was compared with three variants – the left arm relaxed, the left arm simultaneously flexed with a load of 5 kg ("symmetrical" movement), and the left arm simultaneously extended with a load of 5 kg ("asymmetrical" movement).

A total of 600 movements in 5 men was recorded on the inertia dynamometer. The force of the right arm flexions increased an average of 9% when the left arm was simultaneously flexed. The right arm flexion was not as strong when the left arm was simultaneously extended.

These effects appeared more clearly in work with great resistances (power method). The amount of effort in asymmetrical movements increased from day to day, which did not occur in the contraction of symmetrical muscles (see table).

The example given shows that asymmetrical movement could have a slight positive effect on the very first day in one subject, but a negative effect in another, only gradually, after 5 days, becoming positive. This is explained by the difference in individual abilities to master new movements, which is confirmed by the athletic characteristics of the subjects and by careful and systematic observations made on them.

The other indices of muscular activity also changed depending on the experimental variant. When the left arm performed a symmetrical movement, the speed of right arm flexion increased; the time of flexion decreased in comparison with the first variant, i. e., when the left arm was relaxed.

In the third series of experiments, we studied the effect of left arm muscle contractions during fatigue caused by many repeated flexions of the right arm with maximal force. The subject flexed the right arm at the elbow many times, quickly, and these flexions were recorded on the inertia dynamometer. At different times, the work fatigue of the right arm included the left arm, and the subject continued to flex the right arm while the symmetrical muscles of the left arm contracted simultaneously. In all 5 of the subjects, the effort of the fatigued right arm was raised to the original level or higher by the inclusion of the left arm in the work (Fig. 2). The average increase in effort was 13%, reaching 25-30% in individual movements.

Varying the number of left arm muscular contractions and grouping them in a definite order and time of inclusion, we found certain patterns: the positive effect of including the left arm in the work increased with fatigue; the inclusion of the groups containing 5-8 flexions produced the greatest efficiency; the greatest increase

Change in the Force of the Right Arm in 2 Men Resulting From Simultaneous Flexion or Extension of the Left Arm (average effort of 10 movements in percent of the average efforts exerted by the right arm when the left was relaxed)

Day of experiment	1	2	3	4	5	6	1	2	3	4	5	6
When left arm was simultaneously flexed . . .	114	125	110	123	116	121	105	113	107	104	112	112
When left arm was simultaneously extended	101	105	105	114	112	114	98	96	97	98,5	108	110

in the work of the right arm was 4-5% and was obtained when the left arm was included several times in the work (Fig. 2); the effect of including the left arm in the work had a peak-like character (Fig. 2), which, it would appear, reflects the development of successive positive induction at the time.

In the fourth series of experiments, we studied the maximum duration of static effort. The subject held a load weighing from 1.3 to 5 kg in his right arm, which was extended sideways as far as possible. The movement of the loaded arm was recorded directly on a kymograph tape. When signs of fatigue appeared in the right arm (drooping, marked tremor, etc.), at a signal, the subject extended his left arm sideways, or moved his left arm or leg rhythmically. The tension of the left arm or leg muscles prolonged the time that the right arm could hold the load. According to the data from 150 experiments, the average increase in the 5 subjects was 7%. The effect of including the left arm muscles in the activity can be seen in the mechanogram – the right arm, holding the load, was elevated as soon as the left arm joined in the activity; the fluctuation amplitude of the right arm decreased, etc.

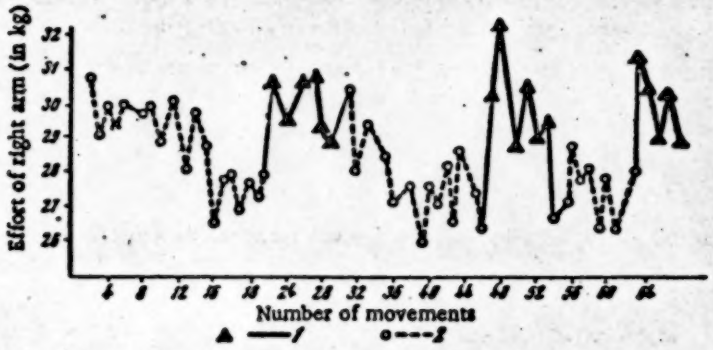

Fig. 2. Effect of contraction of left arm muscles on the efficiency of the right arm in one of subjects.
1) With supplementary flexion of the left arm; 2) without supplementary movements.

In the fifth series of experiments, we studied tension in the upper portion of the body from rhythmic movements of the legs. Sitting in a relaxed pose, the subject turned the pedals of a bicycle ergometer (40-50 pedal turns per minute), then, at a signal, tensed the muscles of the body, grasped the handle more tightly, lightly flexed tensed arms, and clenched his jaws. The result of 70 experiments was that the rate of movement increased an average of 30% in all 7 men during the tension, and remained increased throughout the tension period (Fig.3).

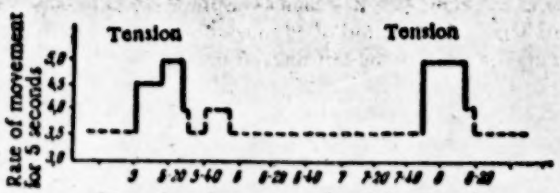

Fig. 3. Change in rotation rate of bicycle ergometer pedals in one of the subjects when the muscles of the upper part of the trunk were tensed.
— With supplementary body muscle tension; -- without supplementary movements.

DISCUSSION OF RESULTS

The data obtained can be considered to be the result of induced influences from one region of the cerebral cortex upon another region. Yet I. M. Sechenov observed that afferent impulses entering nerve centers from single working muscles influence the condition of other muscular group centers. A. A. Ukhtomsky [4] showed that stimulation of single centers can only intensify the stimulation process in other centers when these other centers become a dominating focus. In our experiments, the dominant regulating unit originated during oral instruction, which created a condition of heightened excitability in the motor analysor even before any movement was begun. The afferent impulses, which arose at a definite time due to tension of "supplementary" muscle groups, caused increased stimulation in the dominant focus and an intensified contraction.

Symmetrical movement of the arm produced the greatest effect in the experiments on the inertia dynamometer, since asymmetrical movement first produced a weaker, or negative, effect, which only gradually, with practice, became positive. There can evidently be a negative inducement along with the positive, depending on the kind of movements performed simultaneously.

It is possible that the greatest positive effect is produced by the type of movement combinations found most frequently in labor and athletics, which are fixed conditioned reflexes (symmetrical movement of the arms).

The positive effect obtained by including certain "supplementary" movements in the activity is especially marked on a background of fatigue, which is evidently connected with change in the functional condition of the nerve centers in the cortex of the cerebral hemispheres. Acting through proprioceptors on the central nervous system, one can increase the efficiency of a fatigued body and eliminate, for some time, the advance of fatigue.

SUMMARY

The effect of muscular tension in "supplementary" muscle groups on the efficiency of other muscles was studied in five series of experiments with various forms of muscular activity: muscular contraction, static effort, cyclic movements.

It was established that the inclusion of muscular groups which are not involved directly in the performed movements improves the efficiency of the main movement. The effect was most conspicuous during fatigue. The results were analyzed on the basis of the Sechenov, Pavlov, and Ukhtomsky theories.

LITERATURE CITED

[1] L. L. Vasilyev, G. N. Afanyev and E. E. Plotnikova, in the book: Advances in the Reflexology and Physiology of the Nervous System," Vol. 1, 1926, Coll. 2, pp. 16-30.

[2] N. E. Vvedensky, in the book: The Physiology of the Nervous System," Vol. 3, Book 1, pp. 181-188.

[3] N. E. Vvedensky, in the book: The Physiology of the Nervous System," Vol. 1, 1952, pp. 189-233.

" In Russian.

[4] N. E. Vvedensky and A. A. Ukhtomsky, in the book: Collected Works," Vol. 1, pp. 5-30.

[5] N. N. Goncharov, The Dynamics of Human Muscles with Maximum Tension and Their Change With Age," Dissertation, Moscow, 1952.

[6] S. I. Krapivintseva, Byull. Ekspti.Biol. i Med., 1951, Vol. 32, No. 8, pp. 105-109.

[7] S. I. Krapivintseva, Byull. Ekspti. Biol. i Med., 1951, No. 11, pp. 334-339.

[8] M. E. Marshak, Physical Culture and Sociological Building," 1932, No. 10-11, pp. 52-61.

[9] M. E. Marshak, Labor Hygiene and Safety Techniques," 1936, No. 2, pp. 22-24.

[10] S. P. Narikashvili, and Sh. A. Chakhnashvili, Theoretical and Practical Physical Culture," 1947, No. 7, pp. 317-325.

[11] G. V. Popov, Studies of the West Leningrad University," 1938, No. 23, Article 6, pp. 105-148. 148.

[12] K. S. Ratner, Experiment Studying Static Efforts," Dissertation, Minsk, 1946.

[13] V. V. Rosenblat, Theory and Practice of Physical Culture," 1949, No. 10, pp. 733-740.

[14] I. M. Sechenov, Collected Works," pp. 152-163, Moscow, 1935.

[15] V. S. Farfel, I. M. Freidberg and A. S. Shabashova, Studies in the Physiology of Physical Exercises," pp. 106-113, Moscow, 1939.

[16] D. I. Shatenstein and E. N. Iordanskaya, Fiziol. Zhur. , SSSR, 1955, Vol. 41, No. 1, pp. 35-42.

" In Russian.

ON THE EXCRETORY FUNCTION OF THE STOMACH

A. N. Kuznetsova

From the Physiology Laboratory (Head – Prof. P. K. Anokhin, Active Member of the Academy of Medical Sciences, USSR), the A. V. Vishnevsky Surgical Institute (Director – Prof. A. A. Vishnevsky, Corresponding Member of the Acad. Med. Sci. USSR), Academy of Medical Sciences, USSR

(Received December 23, 1956. Presented by Prof. P. K. Anokhin, Active Member of the Academy of Medical Sciences, USSR)

The excretory function of the stomach is the least studied of all its many different physiological functions.

Researchers from the L. P. Razenkov Laboratory [2, 3, 6, 7, 10] and the M. P. Konchalovsky [4, 8] and R. A. Luriya [1, 5, 9] Clinics have shown the importance and role of the gastrointestinal tract in the excretion processes.

The excretory function of the stomach is most evident and most intense during kidney diseases and other diseases where the body is threatened by the accumulation in the blood and organs of waste substances which are sometimes extremely toxic.

It has also been experimentally shown that artificial increase of these or other substances in the blood is attended by an intensified excretion of them in the stomach.

The purpose of our work was to find whether the excretory function is separate and independent of gastric secretion or if secretion and excretion is a single and united function of the gastric glands.

EXPERIMENTAL METHODS

The experiments were conducted on healthy dogs of about the same age and weight (12-15 kg) with a permanent Basov fistula (10 dogs) or with a Pavlov pouch (2 dogs).

The excretory function was studied on an empty stomach during the action of a food stimulus (meat bouillon) and histamine. To evaluate the excretory function, the dynamics of gastric gland excretion of urea, ammonia and neutral red dye (chromoscopy method) was studied.

Ammonia was determined by Conway's method, urea by the urease method. The animals were kept on a fixed food ration during the whole observation period. The gastric contents were examined for five hours after each hour.

After a "background" excretion of the above products had been established, the animals were intravenously injected with 5-10 ml of 50% urea, prepared in a physiological solution (0.2-0.4 g of urea per 1 kg of animal weight). Neutral red was injected intramuscularly. The threshold concentration of neutral red in the blood, with which the dye, regardless of gastric gland secretion, begins to be excreted by the mucous membrane, can be attained with a 4 ml injection of a 1% (40 mg), freshly prepared, solution of neutral red. The rate of neutral red dye excretion was determined by the time from the moment of parenteral injection to its appearance in the stomach contents.

Neutral red dye concentration in the gastric contents was determined colorimetrically.

The gastric contents were collected through a fistula tube for 4-6 hours, and the amount calculated every 15 minutes. If the contents of the duodenum were observed to reflux into the stomach, examination ceased.

EXPERIMENTAL RESULTS

The stomach began to excrete neutral red dye 5-10 minutes after the dye had been intramuscularly injected. The concentration of the dye in the stomach increased rapidly, reaching maximum concentration (3-4 mg%) after 30-45 minutes. The duration of the excretory period varied in different dogs from 3 hours, 30 minutes to 5 hours. In individual experiments, fluctuations in the excretory process were observed in some dogs, but they were weakly expressed (Table 1). As the amount of dye injected increased, its concentration in the blood increased, and the period of excretion became longer. One such curve for gastric dye excretion is shown in Fig. 1. The maximal concentration of dye in this experiment (3 mg%) was observed $^3/_4$ of an hour after the beginning of excretion. Then the amount of dye excreted began to decrease, and, after 5 hours, only traces of dye could be found in the gastric contents. The total amount of dye excreted was 691γ.

TABLE 1

Neutral Red Dye Excretion by the Gastric Glands on an Empty Stomach

Name of animal	Time in minutes of dye appearance	Time in minutes of maximal dye concentration appearance	Maximal concentration in mg %	Total amount of dye excreted in γ	Total amount of gastric contents in ml	Duration of dye excretion
Ryzhukha	5	30	4.0	334	22.5	4 Hrs. 15 Min.
	8	15	3.0	360	18.0	4 "
Alma	10	45	2.0	322	25.0	3 " 30 "
	10	30	2.0	316	24.5	3 " 45 "
Pushok	5	45	2.0	611	44.5	4 " 30 "
	10	45	3.0	691	49.5	5 "
Bobik	10	30	2.0	408	35.5	3 " 30 "
	10	30	2.0	511	36.0	3 " 45 "
Ryzhik	5	30	2.0	540	41.0	4 "
	5	15	2.0	508	38.5	4 "
Verny	5	30	3.0	367	26.5	4 " 30 "
	10	45	2.0	324	27.0	4 " 15 "

In the experiments studying the effect of the food stimulus and histamine on the excretory function, our purpose was to determine the changes in neutral red excretion during the intensified gastric secretion caused by a single feeding of the dog with meat bouillon (150-200 ml). The results of the experiment showed that the course of dye excretion did not change much during intensified gastric gland secretion. The latent period was unchanged. In separate experiments, excretion was prolonged 30-40 minutes. The dye concentration in the gastric contents hardly changed at all with the increased rate of secretion (almost double) (Table 2).

The dye concentration did not always depend on the amount of gastric juice secreted. A high concentration of the dye was observed both with a large and with a small amount of juice. This, of course, does not negate the fact that the total amount of the substance excreted depends on the amount of juice secreted.

With the parenteral injection of histamine (0.5-1 ml of a 0.1% solution), the excretion of dye with the gastric contents began 3-5 minutes after the injection, but its concentration in each batch did not increase. Under these conditions, the duration of the excretion period was lengthened an average of 15-30 minutes. The total amount of dye excreted increased 2-$2^1/_2$ times in comparison with the total amount of dye excreted on an empty stomach.

In the given case, the amount of dye increased due to the increase in the amount of gastric juice.

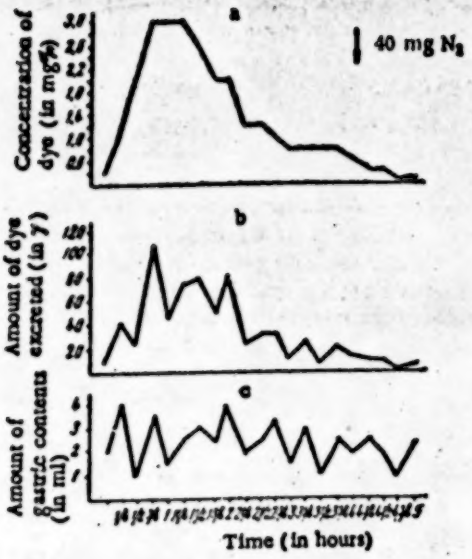

Fig. 1. Excretion of neutral red dye on an empty stomach in the dog Pushok.
a) Concentration of dye; b) amount of dye excreted; c) amount of gastric contents.

In the dogs with Pavlov pouches, the dye was not excreted from the Pavlov pouch on an empty stomach. Dye excretion occurred only after the food stimulus had been given. As in the preceding case, the dye appeared after 5 minutes; its maximal concentration did not exceed 1.6 mg%. The concentration of the dye decreased considerably more rapidly. One must note that dye excretion from the Pavlov pouch was less intensive than from the entire stomach. In this case, the Pavlov pouch evidently does not reflect the excretory function of the stomach as a whole.

According to the data in the literature, dye excretion is known to be mainly carried out by the glands of the pyloric region, and only when they cannot cope with the load do the glands of the fundal region of the stomach participate.

A series of typical patterns also appeared in the excretion of urea and ammonia by the gastric glands.

From 2.7-3.2 mg% of urea and from 6.8 to 7.5% of ammonia were found in the dog's gastric contents. Figure 2 shows the curve characterizing gastric gland excretion of urea and ammonia in a normal animal.

TABLE 2

Effect of Food Stimulus on Neutral Red Dye Excretion

Name of animal	Time in min. of dye appearance	Time in minutes of maximal dye concentration appearance	Maximal concentration in mg%	Total amount of dye excreted in γ	Total amount of gastric contents in ml	Duration of dye excretion
Alma	10	45	3	491	38.5	3 hrs. 30 min.
Bobik	7	15	1.8	608	62	4 hrs. 45 min.
	10	30	3	660	62 5	3 hrs. 45 min.
Pushok	5	30	3	2525	144.5	5 hrs.
	10	45	3	1100	99.5	4 hrs. 30 min.
Ryzhik	5	45	4	1115	75	5 hrs.
	5	30	3	1045	62.5	4 hrs. 45 min.
Sultan	5	45	1.6	692	87	4 hrs.
	5	30	1.6	645	70	4 hrs. 15 min.
Tarzan	5	15	1.6	1003	94	5 hrs.
	5	15	1.6	802	112.5	5 hrs.

Note: In the dogs Sultan and Tarzan – Pavlov pouch; in the other animals – Basov gastric fistula.

While in normal conditions, a small quantity of nitrogenous breakdown products is found in the gastric contents, with urea injected into the blood (0.2-0.4 g per 1 kg of animal weight), a considerable increase in the amount of urea and ammonia in the gastric contents is observed, and there is no doubt that they came from the blood. For example, with an intravenous injection of 5 ml of a 50% urea solution (0.2 g per 1 kg of weight), only a small increase in the concentration and quantity of urea and ammonia was observed. In a majority of cases (experiments on the dog Ryzhik), the maximal concentration of urea appeared during the 5th hour of

observation and consisted of 6.6-12.8mg%. Its concentration did not depend on the amount of gastric juice. A different concentration of urea was observed with the same amount of gastric contents. For example, during the 3rd hour of observation, 6 ml of contents was excreted, and the concentration of urea in the excretion was 6.6 mg% during the 5th hour, the same amount was excreted, but the urea concentration was 4.3 mg%. Analogous data were obtained in the other experiments also. An average of 4.1 mg% of urea was found in the gastric contents.

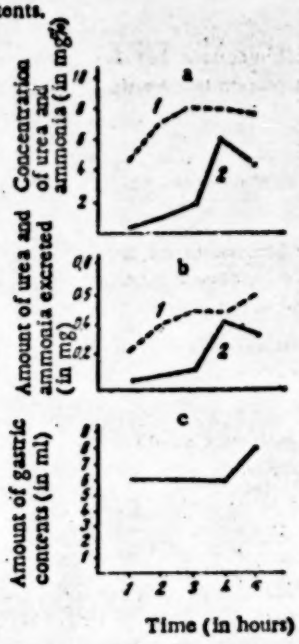

Fig. 2. Gastric excretion of urea and ammonia in the dog Ryzhik. a) Concentration of urea and ammonia; b) amount of urea and ammonia excreted; c) amount of gastric contents: 1) ammonia, 2) urea.

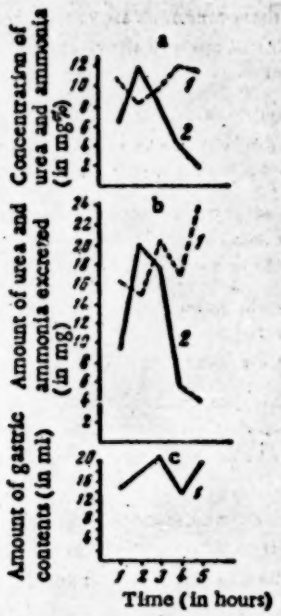

Fig. 3. Gastric excretion of urea and ammonia with supplementary urea injection in the dog Ryzhik, (0.4 g per 1 kg animal weight). Symbols are the same as in Fig. 2.

When 10 ml of a 50% solution of urea was injected into the body (0.4 g per kg of weight), a still greater increase in the concentration of urea was observed; in connection with this, the amount of urea excreted was $2^1/_2$ times the amount excreted in the experiments conducted without the additional urea injection.

The highest concentration was usually observed during the 2nd-3rd hour of observation.

A slightly different picture was observed when ammonia was excreted by the gastric glands. The experimental conditions were the same. When 6 ml of a 50% solution of urea was injected, the concentration of ammonia increased somewhat. The maximal ammonia concentration was from 8 to 15 mg% and appeared, as a rule, near the 5th hour of observation.

The concentration of the substance does not always depend on the amount of gastric juice. For example, in one experiment, 6 ml of juice was excreted with a 6.6 mg% concentration of ammonia during the first hour; during the fifth hour, the same amount of juice was excreted, but with a concentration of 8.0 mg%. Analogous results were obtained in the other experiments. With such a dose, the gastric juice contained 6.5-6.8 mg% of ammonia. When the dose was doubled, the amount of ammonia increased to several times that in the experiments done without the additional injection. The maximal concentration, in most cases, also appeared during the 3rd-4th hour of observation (see Fig. 3). When the injection was increased in the dog Ryzhik, there was about 10.5 mg% of ammonia in the gastric juice (without the additional injection, 6 mg%).

Therefore, with an injected dose of 0.2 g of urea per 1 kg of weight, the amount of urea (3.4-4.1 mg%) and of ammonia (6.5-8.3 mg%) increased. With double the injected quantity (0.4 per 1 kg of weight), a still greater increase in the substances was observed; the urea contained in the gastric juice was 4.5-6.8 mg%, and the ammonia – 10.5-11 mg%.

From our studies, we suggest that the excretory function of the stomach does not depend directly on the secretory function.

Both these functions are closely related, as are all of the gastric functions, but they are independent processes which can proceed simultaneously, or, on the other hand, can change to a known degree independently of each other.

We could not establish any definite dependency of urea, ammonia and neutral red dye excretion on the intensity of gastric secretion. High and low concentrations of these substances can be observed during both increased and decreased gastric juice secretion.

When secretion is intensified, which occurred due to the meat bouillon or the histamine, the amount of gastric juice increases, but the concentration of the substances, as a rule, does not increase. This, of course, does not exclude the dependency of the amount of substance excreted on the amount of gastric juice secreted.

The main general pattern we observed in all of our experiments was that the amount of different substances excreted by the mucous membrane of the stomach principally depends on the concentration of the given substance in the blood.

The rules we observed for urea, ammonia and neutral red dye excretion should not, however, be applied to the excretion of all foreign substances from the blood, as others may be toxic to the stomach wall and may act specifically on its glandular elements.

SUMMARY

The effect of food and histamine on the dynamics of gastric excretion of urea, ammonia and neutral red dye was studied in ten dogs with permanent Basov fistulae, and two dogs with Pavlov pouches. No correlation between the excretion of urea, ammonia, neutral red dye and the intensification of gastric secretion was noted. The amount of substances excreted by the stomach depended mainly on their concentration in the blood.

LITERATURE CITED

[1] Ya. I. Daikhovsky and M. G. Solovei, Terap. Arkh., 1934, Vol. 12, No. 1, pp. 40-51.

[2] K. S. Zamychkina and E. I. Andreeva, Byull. Eksptl. Biol. i Med., 1938, Vol. 6, No. 4, pp. 466-471.

[3] K. S. Zamychkina, and E. I. Andreeva, Fiziol. USSR, 1939, Vol. 26, No. 1, pp. 33-37.

[4] M. P. Konchalovsky, in the book: Works of the 12th All-Union Conference of Therapeutists,* Moscow-Leningrad, 1940, pp. 154-166.

[5] R. A. Luriya, Klin. Med., 1939, Vol. 17, No. 4, pp. 6-14.

[6] I. P. Razenkov, New Data on the Physiology and Pathology of Digestion,* Lecture Moscow, 1948, pp. 240-317.

[7] V. M. Rubel, Material on the Nitrogenous Substances of Gastric and Intestinal Juices in Normal and Pathological Conditions,* Dissertation, Moscow, 1947.

[8] V. N. Smotrov, A. Ya. Zelmanovich, and others, Terap. Arkh., 1936, Vol. 14, No. 4, pp. 527-541.

[9] M. G. Solovei, The Stomach and Kidneys,* Clinical experimental study of the excretory function of the Stomach,* Moscow-Leningrad, 1940.

[10] L. M. Khazen, Byull. Eksptl. Biol. i Med., 1940, Vol. 9, No. 5, pp. 365-367.

* In Russian.

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

EXPERIMENTAL MYOCARDIAL INFARCTION IN CERTAIN PHYSIOLOGIC AND PATHOLOGIC CONDITIONS OF THE BODY

Article II

THE EFFECT OF CIRCULATORY SYSTEM HYPERTONIA ON THE DEVELOPMENT OF EXPERIMENTAL MYOCARDIAL INFARCTION*

S. A. Vinogradov

From the Department of Pathological Anatomy (Head – Prof. A. I. Strukov, Corresponding Member of the Acad. of Med. Sci., USSR), of the 1st Moscow Medical Institute

(Received October 18, 1956. Presented by Prof. A. L. Myasnikov, Active Member of the Academy of Medical Sciences, USSR)

The heart's need of nourishment and of oxygen, particularly, greatly increases when the cardiac muscle is overstrained due to various factors such as great physical strain [4], raised blood pressure, cardiac defects, etc. Overstrain (overloading) of half or all of the heart is often found in myocardial infarction and seems to influence to some degree the development and healing of the infarction.

The functional overloading of the left cardiac ventricle found in hypertonia seems to have an especially unfavorable influence on the development of myocardial infarction. Coronary circulation disturbance is more frequently found, and infarction is more extensive with more serious after-effects in hypertonia patients [9,10, 11,18]. This is partially explained by the sharper atherosclerotic changes found in the coronary vessels and also by the formation of larger round platelets, the heightened permeability of the vascular walls, the frequent ruptures of the platelets, subintimal hematomas and the tendency of the vessels to spasm [1,6,15,16]. There are, however, other factors besides raised blood pressure which affect the heart unfavorably during myocardial infarction.

The purpose of this work was to find how much influence one factor, functional overloading of the myocardium (circulatory system hypertonia), has on the development and the healing of a myocardial infarction.

EXPERIMENTAL METHODS

The experiments were conducted on 56 Chinchilla rabbits, mostly males, weighing an average of 2,000-2,200 g.

A hemodynamic load on the left cardiac ventricle was caused by constricting 2/3 of the abdominal aorta under the diaphragm below the celiac artery origin, and above the orifices of the renal arteries; arterial pressure rose rapidly above the constriction. To disturb coronary circulation, an additional ligature was placed at the descending branch of the left coronary artery, always at the edge of the left cardiac auricle below the origin of the first large right branch, which, in rabbits, goes to the posterior portion of the septum. Ligation of the coronary artery was done either at the same time as coarctation of the aorta or 2-12 days later. In 6 rabbits, the coronary artery was ligated 2 months after coarctation of the aorta during prolonged hypertonia and hypertrophy of the heart. Most of the operations were performed while the animals were under Pentothal anesthesia.

*Presented at the annual scientific session of the 1st Moscow Medical Institute and also at the conference of the Cardiology Department of the Moscow Therapeutic Society.

with no artificial respiration apparatus. To avoid pneumothorax, an incision was made in the center of the thoracic cavity, transecting the sternum.

We measured the blood pressure at various intervals and also took 3 standard and 3 precordial leads of the cardiac contractions and at a distance of 1.5 cm to the right and left. The clearest results were obtained in the precordial leads.

The animals died or were sacrificed by aeroembolism at different intervals in order to study the change in dynamics. The coronary vessels of the heart (in 50% of the cases) were fixed in formalin under pressure, drenched with a contrast medium and examined on stereoroentgenographs. The heart was fixed as a whole in 10% neutral formalin, imbedded in celloidin-paraffin and usually studied on longitudinal (frontal) sections, but also on some lateral, total histo-topographical sections.

The localization and size of the infarctions produced in the experimental animals were marked on cross-section drawings of the anterior and posterior halves of the heart and also on lateral sections, then compared with the control (Fig. 1). Twenty-two rabbits, in which only the descending branch had been ligated, were used as the control.

EXPERIMENTAL RESULTS

After coarctation of the abdominal aorta, the animals' blood pressure usually rose to 130-140 mm of mercury at once and continued to rise rapidly during the days following. On the 2nd-6th day, the pressure was 160-180 mm of mercury, and after 2-3 weeks, 180-200 mm of mercury, sometimes being as high as 220-240 mm of mercury. Long observation (up to 2 months) on some animals showed that the pressure usually remained high the whole time (160, 180, 200 mm of mercury). When the coronary artery was ligated in the hypertonic animals, the pressure fell to 140 and 120 mm of mercury, then rose again.

The electrocardiograms taken the first 2 weeks after coarctation of the aorta almost without exception showed changes in the waves QRS, but these changes were often indefinite and only occasionally related to a turn of the axis to the left (Fig. 2). The decline or inversion of the T waves in the standard and precordial leads was more regular. Analogous results were obtained by V. S. Smolensky [13] and F. Z. Meerson [8]. During artificial coarctation, the T wave changes in the electrocardiogram were evidently caused by the known relative insufficiency of the coronary circulation together with the increased load on the heart.

Fig. 1. Plan of localization and spread of the infarction: a) in control with 1 ligation of the anterior descending artery and b) with ligation when the left cardiac ventricle was functionally overloaded (coarctated hypertonia). 1) anterior half, seen from behind; 2) posterior half, seen from front; 3) lateral section, seen from above.

Three out of the 56 rabbits died on the operating table and 15 at various intervals after coarctation of the aorta.

The morphological changes in the myocardium during the first 2 weeks after coarctation of the aorta consisted in a protein dystrophy of the myocardial parenchyma — a thick swelling of the muscle fiber protoplasm, slight fat dystrophy, some edema of the myocardial stroma, and sometimes small extravasations. Single small foci of myomalacia, with round cell and histiocyte accumulations where the muscle fibers had died, or small proliferations of elongated cells were occasionally observed. F. Z. Meerson [8], in experiments with high (above the valves) constriction of the aorta, observed a sharper myocardial fat dystrophy as well as a considerable decrease in the glycogen concentration in the cardiac muscle during this period.

The anterior descending artery was ligated in the 38 surviving animals. Myocardial infarction was obtained in 32 animals.

Angioroentgenograms showed that the short stump of the ligated branch and the nonligated vessels were well filled with the contrast medium. The myocardial region below the ligature (the basin of the ligated vessel) was only partially filled with the contrast medium through the small anastomoses existing with the other branches of the left coronary artery and with some of the anterior branches of the right coronary artery. These anastomoses and the main trunks of the nonligated vessels were especially well expressed at later intervals after the ligation of the anterior descending artery, i.e., the 2nd and 3rd week. An especially sharp compensatory dilatation was observed in the nonligated, right posterior branch of the anterior descending artery.

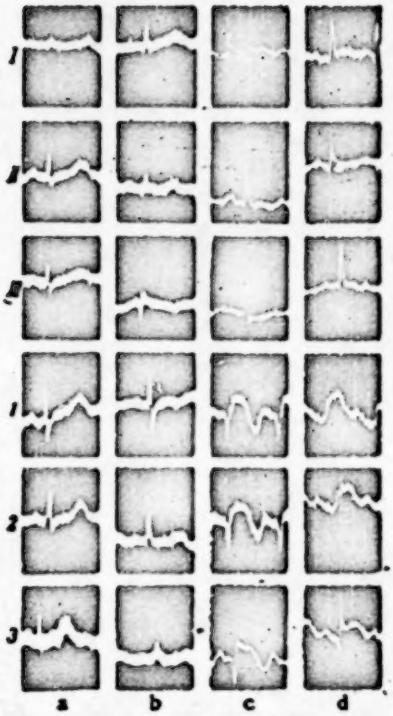

Fig. 2. Electrocardiogram taken in 3 standard and 3 precordial leads; a) original (before operation); b) after coarctation of the aorta and development of hypertonia; c) on the 3rd day after ligation of the anterior descending artery; d) on the 5th day after the development of infarction in conditions of hypertonia. I,II,III – standard leads; 1,2,3 – precordial leads.

In spite of the anastomoses, myocardial infarction regularly developed in the zone of the ligated vessel, and was usually represented by one or several, more or less large sections of myocardial necrosis and profuse cellular infiltration. The infarction was most acute 3-4 days after the coronary artery had been ligated. Subsequently, the connective tissue proliferative reaction predominated and the infarction was gradually healed by resorption of the necrosed sections and scar formation. The spread of the infarction was usually confined in the control animals to the central blood-supply zone of the ligated vessel; the part of the anterior wall of the left ventricle below the ligature, the adjoining portion of the interventricular septum and part of the anterior papillary muscle (see Fig. 1).

The extent of myocardial infarction was considerably greater in the experimental animals than in the control (see Fig. 1). Infarctions of the usual size and localization were only found in 4 of the experimental animals. In 15 rabbits, the infarction was $1\frac{1}{2}$-2 times as great as that in the control, and extended over a large part of the anterior wall of the left ventricle, the anterolateral wall, the anterior papillary muscle and a considerable portion of the interventricular septum. In 10 animals, the infarction was 2-3 times greater than in the control, occupying the entire blood-supply zone of the ligated vessel, including the zone of mixed blood supply and partially invading the sections outside the blood-supply zone of the anterior descending artery: the lateral wall of the left ventricle, the posterior portion of the septum and the posterior papillary muscle (see Fig. 1). In 3 animals, it was difficult to ascertain the degree of infarction spread from morphological data, since death occurred a few hours after the ligation of the coronary artery.

In the central part of the infarction, there were usually one large or several smaller foci of myomalacia surrounded, depending on the interval since the ligation, either by a leukocyte wall, which was well expressed on the 2nd and 3rd days (Fig. 3,a), or by new granulation tissue, which gradually grew into the necrotic zone and matured until the scar stage. The whole process of necrotizing, resorbing and organizing occurred on a background of relative venous congestion, with dilated capillaries, stasis phenomena and small hemorrhages in the infarction region. The contrast medium injected into the coronary system always appeared histologically in the infarction zone, in the small and large arterial vessels, and sometimes in the capillaries. Along the periphery of the infarction, the vessels were especially dilated and filled with the contrast medium.

In a majority of cases, a direct relation was observed between the degree of aortic constriction and the size of the myocardial infarction. However, there was not always a direct relation between the degree of aortic

constriction and the blood pressure level. When the aorta was especially sharply constricted, the blood pressure did not rise, but, on the contrary, fell, and the animals often died. Evidently, the blood pressure level depended on the contractive ability of the myocardium in each specific case, as well as upon the degree of aortic constriction.

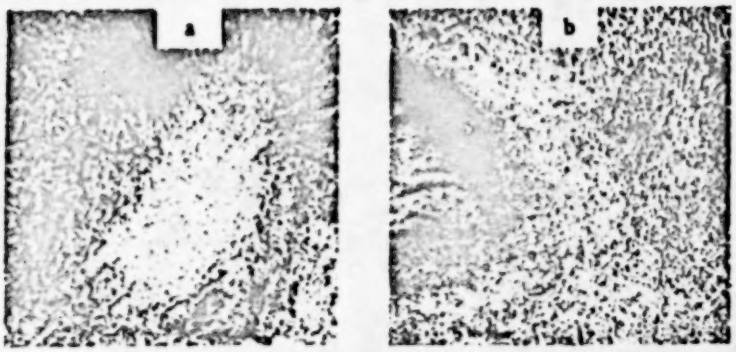

Fig. 3. Histological changes in the myocardium: a) necrosis region in the infarction zone of a control rabbit with one ligation of the coronary artery; leukocyte wall separating the necrosis zone from the surrounding live tissue; b) considerable progression of necrosis beyond the leukocyte wall which had initially separated the first development of necrosis in the myocardium. Capillaries filled with the contrast medium, small necroses and hemorrhages can be seen in the proliferation zone bordering the necrosis.

The greatest spread of the infarction occurred when the coronary artery was ligated three days after the aorta had been constricted, i.e., when the pressure above the artificial coarctation had risen to 160 mm of mercury or more. Therefore, the leading factor determining the size of the infarction, in our experiments, was the degree to which the myocardium was functionally overloaded.

In connection with the infarction spread and, possibly, with the unfavorable conditions created in the myocardium due to its functional overloading, resorption of the necrotic sections and healing of the infarction took a longer period of time than was needed in the control.

In the control animals, resorption and organization of the necrosis foci was observed on the 8th-10th day. In the experimental animals, one could still see large sections of myocardial necrosis in the central zones of the infarction on the 10th day. In some cases, the foci of necrosis remained for 25 days.

In the infarction scar, loose cellular tissue and fatty tissue grew along with the thick collagenous tissue.

The extent of the infarction and the long protraction of the healing process are shown by the changes in the electrocardiographic curve. The electrocardiogram changes were more marked in the experimental animals than in the control. The acute phase of the infarction was also prolonged, then the subacute phase occurred and further restoration of the electrocardiographic curve was retarded. In the control animals, an approximation of the segment ST to the isoline and the appearance of typical coronary waves T were usually observed as soon as the 3rd-4th day after the coronary artery had been ligated, but changes characteristic of the acute and subacute phases of the infarction were still observed on the 6th-7th day in the experimental animals (see Fig. 2).

In 5 experimental animals, a protuberance like an aneurysm formed, with a thinning of the wall on the anterior wall of the left ventricle in the upper half of the heart. According to B. B. Kogan's data [7], a cardiac aneurysm is always formed during the acute stage of the infarction, when the scar has not yet consolidated. We observed an acute aneurysm in 2 animals. In our experiments, evidently, the size of the infarctions, the

protraction of the healing period and the elevated blood pressure (exceeding 20 mm of mercury in 2 rabbits) were the cause. No aneurysms were observed to form in the normotonic control animals.

One must not overlook one more important feature. In 12 animals, signs of the progressing of the process were observed: new circulatory disturbances, hemorrhages, small foci of necrosis along the periphery of the infarction, and new round cell and histiocyte infiltrations. In 4 animals, necrosis spread beyond the limits of the original leukocyte wall (Fig. 3, b). This process progression with new zones of circulatory disturbances, ischemia and myocardial necrosis forming could be traced by the electrocardiographic changes. Newer changes were observed on electrocardiograms taken the 6th-7th day after ligation than on those taken the 3rd-4th day, when the tendency to form the coronary wave T was observed. It seemed as though the acute phase, characterized by a monophasic curve, had recurred (see Fig. 2).

Our data regarding the role of functional overloading of the heart in the development myocardial infarction agree with the data A. I. Smirnov and A. I. Shulgina [12] and with those of A. V. Smolyannikov [14]. These authors showed that circulatory disturbance of the ventricular myocardium due to ligation of the anterior descending artery was much more marked in dogs with vagal stimulation than in the control animals without vagal stimulation or intensification of the cardiac function. A. V. Smolyannikov [14] describes numerous of necrosis foci in both the zone of the ligated vessel and in other regions of the heart. The necrosis foci developed on a background of acute circulatory disturbances and resembled the picture of hemorrhagic infarction, with dilated vessels, stases and hemorrhages. Therefore, when the function is intensified, either by a hemodynamic load or by strong vagal stimulation, sharp coronary circulation disturbances occur, which expand the zone of myocardial ischemia and increase the spread of the myocardial infarction.

Obviously, besides vascular coronary circulation disturbances, the degree to which the myocardium is functionally overloaded is also an extremely important factor, as it determines the localization, spread and healing processes of the myocardial infarction. When local coronary circulation disturbances are present, functional overloading of the myocardium can produce progressive dystrophic and necrotizing processes in the myocardium.

Therefore, myocardial infarctions occurring on a background of hypertonia or attended by a temporary rise in blood pressure should be given special clinical attention.

The experimental data show that, in myocardial infarction cases, one must avoid and guard against both falling and suddenly elevated blood pressure.

There is no doubt that, when determining the condition of a patient with a myocardial infarction in order to prescribe prophylactics and treatment, the question of functional overstrain of the myocardium must be carefully considered.

SUMMARY

In order to evaluate the role of the functionally overloaded left ventricle in the development of myocardial infarction, the anterior descending artery was ligated simultaneously or after artificial hypertension was induced by coarctation of the aorta. Infarction was more prominent and healing and cicatrization were protracted for a longer time in hypertensive than in control animals. In some cases infarction even progressed, involving new portions of the myocardium. At the same time coronary wave T on the electrocardiogram disappeared while the monophasic curve appeared, thus indicating a recurrence of the acute phase.

LITERATURE CITED

[1] N. N. Anichkov and others, in the book: All-Union Conference of Pathologic Anatomists, Leningrad, 1954, Summary of the Proceedings,* pp. 106-112.

[2] S. A. Vinogradov, Arkh. Patol., 1955, No. 1, pp. 76-82.

[3] S. A. Vinogradov, in the book: Annual Scientific Session of the 1st Moscow Sechenov Medical Institute, Summary of the Proceedings,* 1956, pp. 58-59.

[4] S. A. Vinogradov, Byull. Eksptl. Biol. i Med., 1956, No. 5, pp. 27-32.**

* In Russian.

** Original Russian pagination. See C. B. Translation.

[5] S. A. Vinogradov and E. A. Kyandzhuntsova, Works of the 1st Moscow Sechenov Institute," Vol. 1, 1956, pp. 207-215.

[6] K. G. Volkova, in the book: All-Union Conference of Therapeutists, 1956, Summary of the Proceedings," pp. 12-14.

[7] B. B. Kogan and T. S. Zharikova, Klin. Med., 1950, No. 11, pp. 30-41.

[8] F. Z. Meerson, Arkh. Patol., 1954, No. 4, pp. 30-43.

[9] A. L. Myasnikov, Hypertension," Moscow, 1954, pp. 192-196.

[10] G. A. Raevskaya, Sovet. Med., 1948, No. 5, p. 10.

[11] S. Kh. Sidorovich, Sovet. Med., 1955, No. 2, p. 22.

[12] A. I. Smirnov and A. I. Shulgina, Klin. Med., 1955, No. 2, pp. 62-77.

[13] V. S. Smolensky, The Influence of Hypertonia on the Development of Experimental Atherosclerosis," Dissertation, Moscow, 1952.

[14] A. V. Smolyannikov and A. K. Apatenko, in the book: All-Union Conference of Pathologic Anatomists, Leningrad 1954, Summary of the Proceedings," pp. 112-114.

[15] A. I. Strukov and S. A. Vinogradov, in the book: XVI All-Union Conference of Therapeutists, 1956, Summary of the Proceedings," pp. 139-141.

[16] F. I. Yuster, Comparative Morphology of Atherosclerosis in the Coronary Arteries During the Presence and Absence of Hypertension," Dissertation, Moscow, 1954.

" In Russian.

THE EFFECT OF INTRAVENOUS INFUSION OF BLOOD SERUM AND PLASMA FROM HYPERTENSIVE AND NEPHRITIC PATIENTS ON ARTERIAL PRESSURE

S. A. Keizer

From the Department of Internal Medicine (Head – Prof. A. A. Nechaev), Academy of Military Medicine, Leningrad

(Received June 18, 1956. Presented by Prof. A. L. Myasnikov, Active Member of the Academy of Medical Sciences, USSR)

To date, the pathogenesis of hypertension has not been completely explored; further study is particularly needed to clarify the role and importance of the humoral factor. According to data given in the literature, the blood of hypertensive patients contains very small amounts of vasopressive substances. At the same time, however, the data concerning the changes in arterial pressure caused by intravenous infusions of blood, blood plasma and serum from hypertensive patients are extremely contradictory.

V. M. Kogan-Yasny [8,9], Voronov and R. Ya. Spivak [3,4] and others observed arterial pressure to increase in people given an intravenous infusion of blood from hypertensive patients. Pilcring, Fridman and Printsmetal, and others did not observe any peculiarities in arterial pressure change under these conditions.

In experiments on cats and dogs, V. M. Kogan-Yasny, B. A. Vartopetov, R. Ya. Spivak and E. M. Shulman [2,7] observed arterial pressure to increase after an intravenous infusion of blood serum and plasma from hypertensive patients. V. M. Kogan-Yasny [8] and A. K. Gorchakov [5] succeeded in bringing experimental animals out of traumatic and peptone shock by an intravenous infusion of blood serum from hypertonics. R. Ya. Spivak [12] obtained a steady elevation of arterial pressure in rabbits by administering a prolonged intravenous infusion of blood serum from hypertensive patients in a dose of 1 cm^3 per 1 kg of weight.

According to the data of other authors [6,11], the changes in arterial pressure which occur due to an intravenous infusion of blood serum from hypertensive patients are the same as those which occur due to an intravenous infusion of blood serum from normal people. B. F. Angelidze and G. S. Akhmeteli [1] did not obtain any steady rise in arterial pressure by infusing rabbits intravenously with blood serum from hypertonics for a period of 10 days.

The purpose of this work was to investigate and simplify the problem of determining the effect of an intravenous infusion of serum and plasma from hypertensive and nephritic patients on arterial pressure.

EXPERIMENTAL METHODS

Short experiments were done on cats, weighing from 1.5 to 3 kg, under ether anesthesia (28 experiments) and without anesthesia (6 experiments). Arterial pressure was recorded from the femoral artery with a Ludwig manometer on a smoked kymograph tape. Blood serum and plasma from hypertensive patients (23 experiments) and from nephritic patients (4 experiments) were infused into the femoral vein. In the control, the animals were infused with blood serum and plasma from normal people (7 experiments). We usually infused 8-17 cm^3 of plasma and serum, but in a few experiments, 2-5 cm^3.

We also studied the background of arterial pressure and pulse in three dogs for a month. The arterial pressure was measured on a hind paw, by Korotkov's method, before and after the intravenous infusions, which were

given every 5 minutes for 1-3 hours. At first, arterial pressure and pulse were observed after vein punctures (3 experiments) and after the infusion of a physiological solution (6 experiments). Later, when the dogs had almost ceased to react to the vein punctures, the changes in arterial pressure and pulse which occurred after the infusion of blood serum from patients with hypertension (17 experiments), chronic nephritis (2 experiments) and from healthy people (2 experiments) were observed. Each dog was infused with serum 7 times over a period of 10 days in a dose of 0.1 – 3cm^3 per 1 kg of weight each time. The total amount of serum infused was from 5-60 cm^3.

EXPERIMENTAL RESULTS

The arterial pressure of the cats decreased to 30 mm of mercury 10-31 seconds after the intravenous infusion of blood serum, either with or without anesthesia. The marked decline continued for several seconds and then continued, but not so sharply, for 5-7 minutes. Later, the arterial pressure returned to the original level. Only in 6 experiments with the infusion of blood serum both from healthy people and from hypertonics was a slight, temporary rise of 4-8 mm of mercury in the arterial pressure observed, even at the later period. Analogous changes in arterial pressure were observed with the intravenous infusion of blood plasma.

The character of the changes in the cats' arterial blood pressure was the same with the infusion of blood serum and plasma from healthy people as from hypertensive and nephritic patients, and did not depend on the degree of arterial pressure elevation nor on the stage of the disease. Increasing the amount of serum and plasma infused to 17 cm^3 produced a greater decline in arterial pressure, while the infusion of serum in small quantities (2-5 cm^3) caused almost no change in arterial pressure.

When the dogs were intravenously infused with the physiological solution and serum, the greatest changes were in maximal arterial pressure. For example, in 15 out of 19 experiments, a temporary increase of arterial pressure, no more than 20 mm of mercury in 10 experiments, occurred after the infusion of blood serum from people with high blood pressure. But a temporary increase in maximal arterial pressure was also observed in every case where blood serum from healthy people or the physiological solution had been infused.

There was comparatively less change in minimal arterial pressure. When blood serum from hypertensive and nephritic patients was infused, minimal arterial pressure increased temporarily in 9 out of 13 experiments; in 7 experiments, this increase did not exceed 20 mm of mercury. Analogous results were produced by the infusion of blood serum from healthy people and of the physiological solution. There was a slight, temporary increase of arterial pressure in 6 out of the 8 control experiments.

In a few cases, a temporary decline of both maximal and minimal arterial pressure was observed immediately after the infusion of the blood serum. The physiological solution had no such effect.

The pulse remained almost unchanged (frequency increase about 4-10 beats per minute) in the short experiments on dogs, except in the first observations made during the vein punctures.

The picture in all of the 3 dogs was completely different on the 10th day of blood serum infusion. In these experiments, a sharp decline in arterial pressure and retardation of the pulse occurred after the infusion of any amount of the serum. Vomiting, panting and strong salivation began immediately after the serum infusion, with, at first, motorial agitation which subsequently changed into total areactivity. At first, the pulse was hardly noticeable, and the arterial pressure could not be determined. In an hour, all pathologic phenomena had passed, and the arterial pressure and pulse returned to the original level. After such a marked pathologic reaction to the blood serum infusion, the experiments were stopped.

Besides the observations of arterial pressure in the short experiment, we also conducted observations during the whole period of the blood serum infusion and afterwards. There was no tendency to regular arterial pressure increase observed in any of the 3 experiments. On the contrary, it was observed to slightly decrease, but returned to the original level 1-2 days after the serum infusions were stopped. During the entire observation period, the pulse fluctuated within normal limits.

SUMMARY

In short experiments it was shown that the effect of intravenous infusion of blood serum and plasma of hypertensive and nephritic patients upon the arterial pressure of cats and dogs is the same as the infusion of blood serum and plasma of healthy individuals.

• In Russian.

In series experiments after 10 days of intravenous infusions of serum and plasma of hypertensive and healthy individuals, arterial pressure of dogs was almost unaltered, and only a slight tendency of lowering was noted.

LITERATURE CITED

[1] B. F. Angelidze and G. S. Akhmeteli, in the book: Works of the II Enlarged Scientific Session of the Institute of Cardiology on the Problem of Hypertension,* Tbilisi, 1953, pp. 393-397.

[2] B. A. Vartapetov, R. Ya. Spivak and E. M. Shulman, Byull. Eksptl. Biol. i Med., 1942, Vol. 5-6, No. 11-12, pp. 41-44.

[3] Ya. K. Voronov and R. Ya. Spivak, Klin. Med., 1941, Vol. 19, No. 1, pp. 95-100.

[4] Ya. K. Voronov, Vrach. Delo, 1946, No. 11-12, pp. 915-920.

[5] A. K. Gorchakov, Vrach. Delo, 1945, No. 3-4, pp. 119-124.

[6] L. N. Karlik and N. I. Burachevsky, Klin. Med., 1945, No. 10-11, pp. 52-56.

[7] V. M. Kogan-Yasny, B. A. Vartopetov and R. Ya. Spivak, Byull. Eksptl. Biol. i Med., 1937, Vol. 2, No. 4, pp. 91-95.

[8] V. M. Kogan-Yasny, Byull. Eksptl. Biol. i Med., 1946, Vol. 9, No. 4, pp. 214-217.

[9] V. M. Kogan-Yasny, Vrach. Delo, 1949, Vol. 26, No. 11-12, pp. 793-800.

[10] V. M. Kogan-Yasny, Byull. Eksptl. Biol. i Med., 1942, Vol. 14, No. 2, pp. 25-27.

[11] V. A. Lebedeva and A. Ya. Yaroshevsky, Byull. Eksptl. Biol. i Med., 1950 Vol. 30, part 5, No. 11, pp. 315-320.

[12] R. Ya. Spivak, Vrach. Delo, 1946, No. 11-12, pp. 841-846.

* In Russian.

A STUDY OF THE FUNCTIONAL CONDITION OF THE THYROID GLAND DURING EXPERIMENTAL ATHEROSCLEROSIS

N. N. Kipshidze

From the Institute of Therapy (Director – Prof. A. L. Myasnikov, Active Member of the Academy of Medical Sciences, USSR), Academy of Medical Sciences USSR, Moscow

(Received October 28, 1956. Presented by A. L. Myasnikov, Active Member of the Academy of Medical Sciences, USSR)

The works of our own and of foreign scientists have proven that the thyroid gland plays an essential role in the development of atherosclerosis. In an experiment with animals, it was established that an injection of the thyrotropic hormone inhibits the development of experimental atherosclerosis [3,4]. But, when injected simultaneously with cholesterol thiouracil (a substance which reduces the efficiency of the thyroid gland), it sharply intensifies the atherosclerosis development [1,5].

That atherosclerosis develops early and extensively in myxedema and that it develops weakly in thyrotoxicosis patients are known facts.

The purpose of our work was to study the functional condition of the thyroid gland during experimental cholesterol atherosclerosis.

EXPERIMENTAL METHODS

We studied the function of the thyroid gland by means of radioactive iodine. Atherosclerosis was produced by N. N. Anichkov's method.

The experiment was done on 21 mature rabbits of the same type, weighing from 2 to 2.3 kg, kept in the same conditions and receiving the same food ration. Before the experiment began, the cholesterol content in the blood was determined in all of the rabbits by the Grigo method, and the functional condition of the thyroid gland was studied by means of radioactive iodine. We used iodine with an atomic number of 53 and an atomic weight of 131 (I^{131}).

The disintegration period of I^{131} is 8 days. I^{131} was injected subcutaneously into all the rabbits in a dose of 0.75-1.0 microcuries and, after 24 hours, when a Geiger counter count had been made, the figures obtained of I^{131} absorption were expressed in percentages. After thyroid gland absorption of I^{131} had been determined, cholesterol dissolved in vegetable oil (0.3 g of cholesterol per 1 kg of animal weight) was administered daily to 18 rabbits, for a period of 6 months. To exclude the effect of the vegetable oil on the function of the thyroid gland, 3 rabbits were given daily for 6 months the same amount of vegetable oil at the same time as the other 18 rabbits, but without the cholesterol.

The cholesterol content in the blood was determined in all of the rabbits every 15 days. After 6 months, the thyroid gland absorption of I^{131} was again examined in all of the rabbits, after which the rabbits were sacrificed, the aorta and heart extracted and the aorta stained in toto with Sudan III; the heart was cut into a series of consecutive sections, the fat was stained with Sudan III and the degree of atherosclerosis in the coronary arteries was examined. We arbitrarily designated the degree of atherosclerotic changes in the aorta and coronary arteries thus: + weak, ++ moderate and +++ sharp changes.

EXPERIMENTAL RESULTS

Before cholesterol and vegetable oil ingestion started, I^{131} absorption was 15-27% in all of the rabbits.

The cholesterol content in the blood increased rapidly after cholesterol administration began in most of the rabbits and, by the end of the first month, had reached 620-680 mg%, while the cholesterol content in the blood remained within normal limits (20-96 mg%) throughout the experiment in the rabbits which were receiving vegetable oil alone.

In most cases, hypercholesteremia reached 1,100-1,400 mg% 3-4 months after the beginning of cholesterol administration, and, in 2 rabbits, 1,580 and 1,730 mg%. In two other rabbits, however, the blood cholesterol content never exceeded 470-600 mg% throughout the period of cholesterol administration. Towards the end of the 6th month of cholesterol ingestion, the cholesterol content in the blood was considerably lower, in some cases, 320-422 mg%.

When thyroid gland absorption of I^{131} was examined for the second time, after 6 months of cholesterol ingestion, it was found to be greatly reduced in all 18 rabbits and was, in some cases, 0.10-0.22% instead of the original (before cholesterol ingestion began) 15.4-20.0% (rabbits No. 9,10,15, Table 1).

TABLE 1

Thyroid Gland Absorption of I^{131} in Connection with Hypercholesteremia and Atherosclerosis of the Aorta and the Coronary Arteries

No. of rabbit	Thyroid gland absorption of I^{131} in %		After 6 months period of cholesterol ingestion	Cholesterol content in blood at end of 6 month period in mg%	Degree of atherosclerosis of the aorta	Degree of atherosclerosis of the coronary arteries
	before cholesterol ingestion	after 6 month period of cholesterol ingestion				
1	24.1	13.2	470	320	++	+
2	19.5	5.2	1212	1030	+++	++
3	17.9	4.25	1340	1136	+++	++
4	18.2	4.8	1280	1025	+++	++
5	16.3	3.88	1320	714	+++	++
6	17.2	5.1	865	612	+++	++
7	20.7	6.2	1270	615	+++	++
8	19.5	8.2	1110	422	++	+
9	19.1	0.22	1580	1124	+++	+++
10	15.4	0.10	1730	1240	+++	+++
11	22.1	6.4	950	618	+++	++
12	22.3	6.55	912	518	+++	+
13	21.2	4.5	1370	816	+++	++
14	27.6	8.7	780	604	++	+
15	20.0	0.22	1400	1010	+++	++
16	25.1	11.6	600	410	++	+
17	24.2	3.8	1450	1116	+++	++
18	22.1	4.2	1380	810	+++	++

Upon examination of the aorta and the coronary arteries, acute atherosclerosis of the aorta and moderate atherosclerosis of the coronary arteries were observed in most cases.

We observed no atherosclerosis of either the aorta or the coronary arteries in the rabbits which had received vegetable oil without cholesterol. In these rabbits, thyroid gland absorption of I^{131} remained within normal limits after the 6 month period of vegetable oil ingestion (Table 2).

Consequently, vegetable oil without cholesterol does not affect the function of the thyroid gland.

When I^{131} absorption is compared with the high hypercholesteremia and the degree of atherosclerosis in the aorta and coronary arteries, the relation between the thyroid gland absorption of I^{131} and the degree of atherosclerosis is obvious (Table 1). In those cases when I^{131} absorption was sharply reduced, acute hypercholesteremia and atherosclerosis of the aorta were observed. When I^{131} absorption was not so markedly reduced (rabbits No. 1,8,14,16), hypercholesteremia and atherosclerosis of the aorta and coronary arteries were less acute. V. V.

Tatarsky [2] who examined the basal metabolism in rabbits with experimental atherosclerosis, also discovered that basal metabolism in animals with high hypercholesteremia and marked atherosclerosis declined sharply.

TABLE 2

Thyroid Gland Absorption of I^{131} in Rabbits which Received Vegetable Oil Without Cholesterol

No. of rabbit	Thyroid gland absorption of I^{131} in %		Cholesterol content in blood before vegetable oil ingestion in mg%	Cholesterol content in blood after 6 month period of vegetable oil ingestion in mg%	Degree of atherosclerosis of aorta	Degree of atherosclerosis of coronary arteries
	before vegetable oil ingestion	after 6 month period of vegetable oil ingestion				
19	18.7	17.6	78	64	—	—
20	21.7	20.4	65	82	—	—
21	17.8	17.1	76	86	—	—

Therefore the function of the thyroid gland is weakened in cholesterol atherosclerosis, and the weaker the glandular function, the stronger the atherosclerosis.

Thyroid gland absorption of I^{131} depends directly on the level of hypercholesteremia and the degree of atherosclerosis.

The ingestion of vegetable oil without cholesterol does not cause hypercholesteremia and atherosclerosis to develop and does not affect thyroid gland absorption of I^{131}.

SUMMARY

The functional state of the thyroid gland of rabbits with experimental atherosclerosis of the aortas and coronary arteries was studied with the aid of I^{131}.

After six months of cholesterol ingestion, absorbtion of I^{131} decreased from the initial 15.4-20.0% to 0.10-0.22%. Under conditions of hypercholesterinemia and atherosclerosis, absorbtion of I^{131} diminished and the functional state of the thyroid gland degraded.

LITERATURE CITED

[1] T. A. Sinitsina, Doklady Akad. Nauk SSSR, 1953, Vol. 93, No. 5, pp. 929-932.

[2] V. V. Tatarsky, Arkh. Biol. Nauk SSSR, 1940, Vol. 60, No. 3, pp. 29-37.

[3] V. V. Tatarsky, and V. D. Tsinzerling, Arkh. Patol., 1950, Vol. 12, No. 1, pp. 44-55.

[4] L. B. Friedland, Ztschr. f. d. ges. exp. Med., 1933, Bd. 87, S. 683-702.

[5] A. Steiner and F. E. Kendall, Arch. Path., 1948, Vol. 42, No. 4, pp. 433-444.

THE CHARACTER OF EXPERIMENTAL ANEMIAS CAUSED BY DENERVATING VARIOUS SECTIONS OF THE DIGESTIVE TRACT

R. A. Durinyan

From the Institute of Normal and Pathologic Physiology (Director – Prof. V. N. Chernigovsky, Active Member of the Acad. of Med. Sci., USSR), Academy of Medical Sciences, USSR

(Received October 16, 1956. Presented by Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

The existence of a close functional link between the organs of the alimentary canal and the blood system is widely recognized today. It has been shown that the exclusion of the gastrointestinal tract receptors and influences on them changes the composition of the blood [1,2,3,4,5,10,12]. The studies establishing the development of anemia due to denervation of certain internal organs are especially interesting [5,6,7,8,10,11].

The purpose of our work was to study the general and specific pictures of anemias caused by denervating the caudal section of the small intestine, the duodenum and a portion of the stomach.

EXPERIMENTAL METHODS

Cats and dogs (males) were studied as long-term experiment. One group was used as the control and in the experimental animals, one of the above organs was denervated. The amount of erythrocytes, hemoglobin, leukocytes, reticulocytes, osmotic fragility and the average diameter of the erythrocytes and the hematocrit index were determined in all of the animals. Bone marrow punctates were examined in some of the animals. The amount of bilirubin and easily-liberated iron were also determined in the dogs, and studies were done with labeled iron.

All the operations were performed under general ether anesthesia. When denervating the duodenum and the caudal section of the small intestine, all the nerves running through the part in question were resected. The adventitia was also removed from the vessels, and the vascular walls were coated with a 10% phenol solution.

The stomach was denervated by forming a pouch according to Klementsievich and Heidenhain's method (of the usual size), thus denervating the bottom and part of the body of the stomach.

Each animal was examined for 4-10 months (5-7 months average).

EXPERIMENTAL RESULTS

After a section 80-100 cm long of the caudal section of the small intestine had been denervated, anemia with a typical intermittent course developed in the cats. The first weeks after denervation, the anemia followed a hypochromic course and was attended by increased reticulocytosis with a shift to the left (Fig. 1). The changes in osmotic fragility and average erythrocyte diameter suggest that anemization is associated with some intensification of hemolysis.

A few "anemic waves", which could be classified as normochromic, normocytic regenerative anemia, usually occurred after the postoperative anemia. There were no signs of intensified blood hemolysis at that time (see Fig. 1). Therefore, denervation of the caudal region of the small intestine causes definite disturbances in the blood system. The functional disturbance of the denervated intestinal section is probably not of

great importance in this context, since, in experiments in which this section of the intestine was resected, only a slight, temporary (3-4 weeks), hypochromic anemia was observed to develop.

Therefore, denervation caused greater change in the blood composition than did resection of the same intestinal section.

More substantial changes were observed in the blood and bone marrow after denervation of the duodenum.

In this case, the anemia was also intermittent in character. During the first period, the number of erythrocytes decreased by 2 million (Fig. 2), and the hemoglobin percentage was reduced at the same time. The anemia was hypochromic. The amount of reticulocytes greatly increased, erythrocyte osmotic fragility changed and microspherocytosis appeared. Erythropoiesis was noticeably intensified (Table 1), which indicates intensified hemolysis. During the second anemic wave, which was classified as hyperchromic, macrocytic and hyporegenerative, a further reduction in the number of erythrocytes occurred. The color index rose above 1. The number of reticulocytes decreased; erythropoiesis was suppressed (see Fig. 2, Table 1). There were no signs of intensified blood hemolysis.

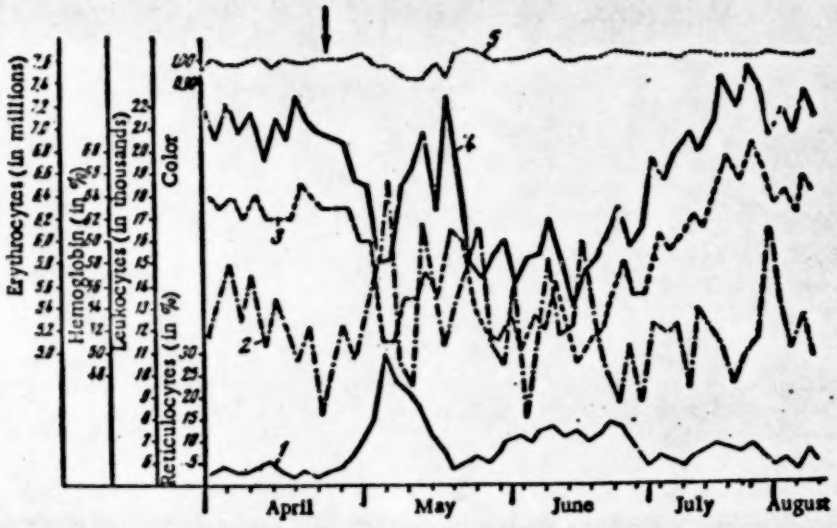

Fig. 1. Change in peripheral blood after denervation of the caudal region of the small intestine.
1) reticulocytes; 2) leukocytes; 3) hemoglobin (according to Sahli); 4) erythrocytes; 5) color index; arrow shows the day of the operation.

Therefore, denervation of the duodenum led to considerable and lasting charges in the blood composition and to erythropoiesis suppression.

It could be suggested that the destruction of the efferent links when the duodenum is denervated could cause the synthesis of the antianemic factor in the mucous membrane of the duodenum to be disturbed. This, however, is not sufficient explanation for the development of such considerable changes in the blood, since the antianemic activity of the duodenum is 3 times less than that of the stomach or of the rest of the intestine.

In the control animals (preparation of the vessels and nerves of the duodenum), a slight, tempory (3-4 weeks) anemia, with some intensification of hemolysis, was observed.

Anemia with the typical intermittent course also developed when the abdominal section of the dogs with a pouch according to Klementievich and Heidenhain) was denervated.

During the first period, the amount of erythrocytes and hemoglobin sharply decreased, and the reticulocytes

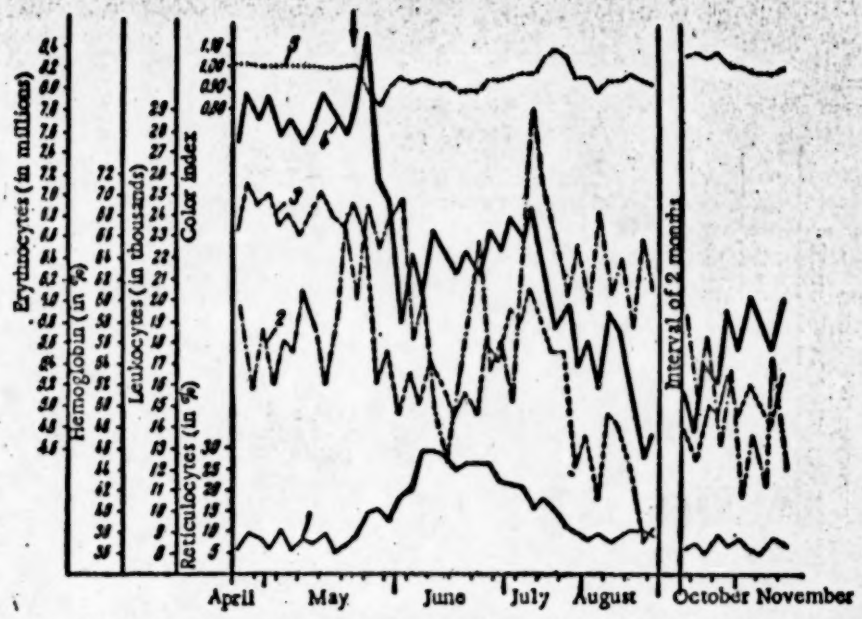

Fig. 2. Change in peripheral blood after denervation of the duodenum. Symbols are the same as in Fig. 1.

TABLE 1

Change in Erythrogenic Portion of Bone Marrow after Denervation of the Duodenum in Cats (in percent of 100 bone marrow cells)

Cat	Date of examination	Period of examination	Amount of dividing forms (mitoses)	Erythroblasts	Pronormoblasts	Normoblasts: Basophilic	Normoblasts: Polychromatophilic	Normoblasts: Orthochromatic	Reticulocytes	Number of erythrocytes per 1 cu mm peripheral blood (in thousands)
№ 18	14/V	Before operation	0.3	0.6	1.1	5.1	11.3	15 7	0.9	7660
	4/VIII	After operation	0.2	1.0	1.3	5.7	9.6	13.1	1.0	5190
	28/XI		—	1.3	1.9	4.2	8.1	9.0	0.6	6000
№ 17	18/V	Before operation	0.2	0.7	1.4	6.3	13.4	17.0	1.1	7980
	12/VIII	After operation	—	1.2	1.9	4.5	10.0	10.8	0.6	5840
№ 20	7/V	Before operation	0.4	0.7	1.3	5.8	13.7	16.9	0.9	7240
	8/VIII	After operation	—	1.3	2.2	3.6	11.2	11.7	0.6	5710

increased to 5 times the original number (Fig. 3). Also, change in fragility was observed, the average diameter decreased and microspherocytosis appeared. The amount of bilirubin and easily-liberated iron increased. Digestive tract absorption and erythrocyte assimilation of Fe^{59} increased considerably. However, Fe^{59} quickly disappeared from the erythrocytes.

All these signs point to increased hemolysis of the blood.

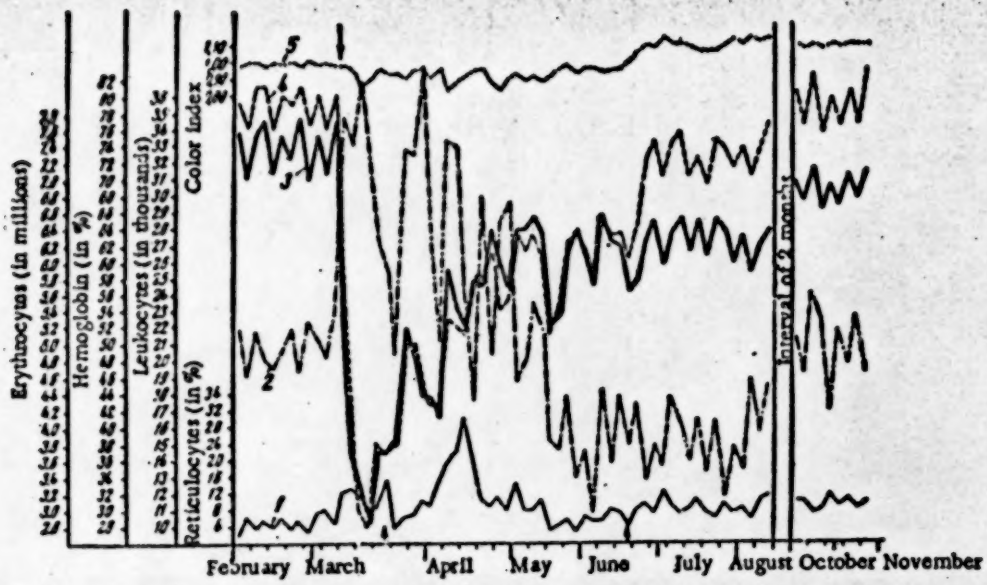

Fig. 3. Change in peripheral blood picture after formation of a pouch in dog Merry according to Klementsievich-Heidenhain. The arrows show radioactive iron administration. The other symbols are the same as in Fig. 1.

The postoperative anemia (Table 2) was classified as the hypochromic, microcytic and regenerative type.

Two months after the operation, the amount of hemoglobin and erythrocytes had increased, but had not yet regained the original level. The erythrocyte deficiency was 1-1.5 millions, which was retained throughout the observation period. The color index rose above 1. The amount of reticulocytes decreased (Fig. 3). Osmotic fragility returned to the original level, and macroplanocytosis appeared. The amount of bilirubin decreased, and the iron again appeared in abundance. The intensity of Fe^{59} absorption and assimilation became almost normal, and the duration of erythrocyte life increased. Erythropoiesis was noticeably suppressed (Table 2).

TABLE 2

Change in Erythrogenic Portion of Bone Marrow after Formation of a Pouch According to Klementsievich-Heidenhain in the dog Merry (in per cent of 100 bone marrow cells)

Examination period	Date of examination	Amount of division forms (mitoses)	Erythroblasts	Pronormoblasts	Normoblasts			Reticulocytes	Number of erythrocytes per 1 cu mm of peripheral blood (1000s)
					Basophilic	Polychromatophilic	Orthochromatic		
Before operation	26/II	0.3	0.6	1.2	6.1	9.2	13.4	0.9	7720
After formation of miniature stomach	23/III	0.4	0.8	1.4	6.7	9.1	13.0	1.8	3830
	22/IV	0.3	0.7	1.4	6.6	9.7	14.2	1.1	6340
	29/VI	0.2	0.9	1.5	7.2	10.8	11.1	0.8	6440
	13/VII	0.1	0.8	1.7	7.9	11.3	10.0	1.2	6320
	10/XI	—	0.8	1.6	8.3	11.8	8.3	1.1	6950

Therefore the anemia was already the hyperchromic, macrocytic, hyporegenerative type.

Consequently, in the long run, denervating a portion of the stomach caused erythropoiesis suppression, which reduced the antianemic activity of the juice from the pouch [9, 11].

However, the pouch was excluded from the digestive processes and did not participate in vitamin B_{12} assimilation; therefore the disturbance of the antianemic factor synthesis in the pouch could not lead to erythropoiesis disturbances. Moreover, in the dogs the pyloric portion of the stomach had a larger antianemic activity than the fundal and, finally, the intestine had a considerable antianemic activity.

Postoperative, hypochromic anemia, characterized by some signs of blood hemolysis intensification, developed in the control dog with a Pavlov pouch. After a month, the blood picture returned to normal.

Postoperative, hypochromic anemia, caused by increased hemolysis, was observed to develop after all our operations, regardless of their nature or localization.

DISCUSSION OF RESULTS

Therefore, denervating various sections of the digestive tract leads to the development of anemias, distinguished by known features. To understand the mechanism of such anemias, one must keep in mind that their first period was characterized by the more or less general traits typical of the anemias which attended the control operations. Evidently, during the first period, the anemias do not have specific features and are an after-effect of operational trauma. It is possible that denervation anemia has a dual mechanism; it is formed from the reaction of the body to the loss of blood and from the reaction to the nervous system trauma caused by the transection of many nerve trunks. In the first period, the anemias are classified as hemolytic.

Then, in the subsequent period, the anemia acquires the specific features described above.

How is it possible, proceeding from factual data, to describe the mechanism of these anemias? They cannot be associated with the decreased function of a given organ or a part of same, since the removal of the spleen or the resection of part of the intestine do not cause anemia like that observed after denervation.

The best way of describing the mechanism of the anemias observed is that they result from the simultaneous blocking of both the afferent and efferent links. The exclusion of these links and others cause the organ, or a section of it, to leave the control of the nervous system; the sections of the central nervous system do not receive the necessary signaling from the periphery and cannot change the organ's operation in the required direction, due to the sectioning of the efferent nerves.

This explanation is based on the theory expressed by V. N. Chernigovsky and A. Ya. Yaroshevsky [10] that the stability of the blood composition is maintained by the constant tonic impulses going from the organs to the central nervous system. The tonic impulses, in their turn, are necessary to realize the efferent, regulating action of the nervous system on the hematopoietic apparatus. Recently, E. Komla published a monograph [13] introducing the view that, in the organs which have to do with hematopoiesis, constant formation of the special substances "poietins" occurs and these substances maintain the normal level of hematopoiesis. Denervation of these organs stops the formation of these substances.

Such an explanation does not negate, but rather supports the thought that the nervous system is probably important to the maintainance of normal activity in the blood system organs. Evidently, "poietins" are only the intermediate humoral link in nervous system regulation of the blood system.

SUMMARY

Studies of the blood picture and bone marrow before and after denervation of various parts of the alimentary canal in animals in series experiments showed that denervation of the caudal part of the small intestine caused the development of a normochromic normocytic anemia; denervation of the duodenum—hyperchromic macrocytic anemia, and denervation of a part of the stomach led to hyperchromic macrocytic anemia.

* In Russian.

LITERATURE CITED

[1] Ts. L. Abakeliya, Reflex Effects from the Stomach on the Composition of Peripheral Blood in Certain Forms of Anemia," Author's abstract of Dissertation, Tbilisi, 1955.

[2] G. V. Ageev, in the book: Works of the Saratov Medical Institute Institute," 1939, Vol. 2, No. 2-3, pp. 25-37.

[3] D. L. Goldberg, Essays on Hematology," Tomsk, 1952.

[4] A. Ya. Gubergrits, Vegetative Regulation of the White Blood Cells," Kiev, 1941.

[5] E. L. Kan, Data on the Study of the Nervous System's Influence on Blood Composition," Leningrad (1953).

[6] O. L. Moiseeva, On the Share of the Liver in the Regulation of Blood Composition," (Clinical-experimental study).

[7] O. M. Preger, in the book: Works of the III Pavlov Conference," Tomsk, 1953, pp. 58-61.

[8] O. M. Preger, in the book: Works of the IV Pavlov Conference," Tomsk, 1954, p. 67.

[9] N. A. Fedorov, in the book: Contemporary Problems of Hematology and Blood Transfusion," 1955, No. 31, pp. 75-77.

[10] V. N. Chernigovsky and A. Ya. Yaroshevsky, Problems of Neural Blood System Regulation," Moscow, 1953.

[11] S. L. Yakovlev, Dissertation," Leningrad, 1955.

[12] A. Ya. Yaroshevky, Material on the Interoception of the Bone Marrow and the Interoceptive Influence on Blood Composition," Leningrad, 1948.

[13] E. Komla, Die Zentralnervöse Regulation des Blutbildes, Stutgart, 1956.

" In Russian.

THE EFFECT OF PANCREATIC DENERVATION ON THE BLOOD SYSTEM

V. I. Teterina

From the Department of Pathophysiology (Head – Prof. D. I. Goldberg), the Tomsk Medical Institute

(Received October 30, 1956. Presented by Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

The stimulation of different receptor fields or the exclusion of receptor zones leads to marked changes in the blood system.

Methods of denervating various internal organs were worked out in the laboratory of V. N. Chernigovsky. These methods made it possible to study the reaction of the blood system to the denervation of the carotid sinuses and the arch of the aorta [1,5,6,10,12], the spleen [2,3,4,9], the liver [7,8], and different sections of the stomach and intestines. Rather profound and lasting anemia was obtained in all of the experiments, which originated from erythropoiesis disturbance, with adisturbance of the erythroblastic maturing processes which developed long after the operation.

Therefore, the organs of the blood system are connected with the central nervous system by a bilateral nerve link. V. N. Chernigovsky and A. Ya. Yaroshevsky [11] advanced the possibility that there are two mechanisms regulating the activity of the blood system organs.

On the one hand, the activity of the blood system organs can be stimulated by impulses coming from the peripheral blood (in connection with the change in its chemical composition and physicochemical properties) into the central nervous system, from which place centrifugal impulses proceed to the executive organs - the blood system organs.

On the other hand, the activity of the blood system organs can also be stimulated reflexively, but the reflex originates from the receptor fields of the various organs sending impulses to the central nervous system, from which signals proceed to the blood system organs.

Therefore, on the basis of many factual data, many researchers have concluded that the exclusion of individual blood system organs and, also, of other receptor fields is reflected in the peripheral blood picture.

The effect of pancreatic denervation on the blood system has not yet been treated in the literature.

The purpose of our work was to trace the hematological changes occurring due to denervation of the pancreas.

EXPERIMENTAL METHODS

Seven healthy dogs with good hematological indices were used for the experiment. The pancreas was denervated in three of the dogs, and the other dogs were used as the control.

Denervation was done by carefully separating the organ from the surrounding tissues. The adventitia was carefully scraped from the main large 5-6 vascular trunks feeding the pancreas and were then coated with a 10% phenol solution over a section 1-1.5 cm long. The pancreatic ducts were processed in the same manner. The small vessels were ligated from two directions and transected.

For two weeks before the operation, the background blood composition was examined for hemoglobin content and number of erythrocytes, reticulocytes and leukocytes; myelograms and hemograms were examined, and the erythrocyte diameter and blood sugar content on an empty stomach were determined. After the operation, all the blood indices were examined after 3-4 days, and then every 7-10 days; every 15 days, a bone marrow punctate was studied, and the erythrocyte diameter was measured. The final stage was a microscopic study of the denervated pancreas.

EXPERIMENTAL RESULTS

After the operation, anemia developed in the dogs, with a maximal hemoglobin decrease of 13-20% by the 8th-24th day. After the 30th day, the hemoglobin content began to increase in all of the animals and, in Maryak, reached the original level on the 84th day, in Bars, on the 47th day and, in Laska, on the 66th day. Analogous changes were observed in the erythrocytes; the maximal decrease was 1.8-2.3 millions on the 8th-24th day of the experiment, then they began to gradually increase and reached their normal level on the 40th-66th day. The reticulocyte reaction was absent in all cases, and the white blood picture was essentially unchanged.

The study of bone marrow hematopoiesis showed that the principal changes in the myelogram concerned the polychromatophilic normoblasts, which increased 5-14% in number; the leuko-erythroblast ratio consequently decreased from 1.2-1.5 to 0.8-0.9. These changes indicated hyperplasia of the erythropoietic tissue and some disturbance of the maturing process of the cellular elements.

The average erythrocyte diameter was slightly changed; only in one dog was there an increase of some 0.25 micron. No shift of the peak in the Price-Jones curve was observed.

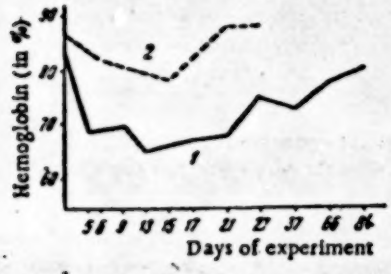

Fig. 1. Dynamics and time of Hemoglobin restoration in Maryak and Malchik.
1) pancreas denervated Maryak); 2) pancreatic ducts ligated (Malchik).

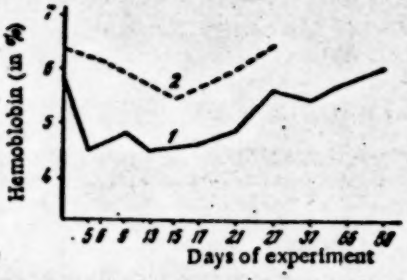

Fig. 2. Dynamics and time of erythrocyte quantity restoration in Maryak and Malchik. The symbols are the same as in Fig. 1.

At first, the blood sugar stayed within the limits of physiological fluctuations, but, after the 13th-27th day, there was a short period (10-14 days) in which its level decreased to 58-64 mg%, after which the sugar content returned to normal.

In the control group of dogs, the gland was totally resected (depancreatization), the pancreatic ducts were ligated or only laparotomy was done, with cauterization of the peritoneum by a 10% phenol solution.

Comparative analysis of the data obtained showed that the hematological indices were more quickly restored in the control than in the experimental group: maximal hemoblobin reduction was 8-20% on the 6th-15th day and maximal erythrocyte reduction, 0.8-1.5 million; the indices were restored on the 18th-21st day, but they were not restored in the experiment until the 47th-84th day (Fig. 1,2). In the control with the laparotomy, the reduction of the indices was extraordinarily weak (hemoglobin: 2-3%, erythrocytes: 0.2-0.3 million), and restoration was observed after only a week.

During the operation, the vessels were not carefully separated nor the adventitia scraped off in the dog Bars; it is interesting to note that the vessels were coated with phenol while still covered by cellular tissue. Restoration of the hematological indices occurred earlier in this dog (on the 47th day) than in the other two (on the 66th-84th day), but much later, however, than in the control animals (on the 18th-21st day) (Fig. 3,4 and Table).

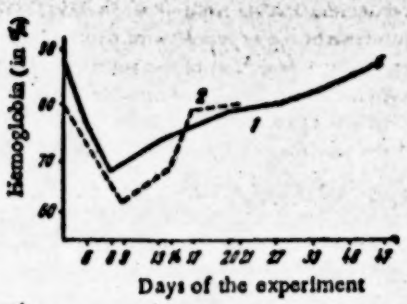

Fig. 3. Dynamics and time of hemoglobin restoration in Bars and Bobka.
1) pancrease denervation (Bars); 2) total resection of the gland (Bobka).

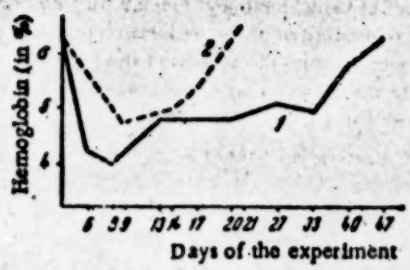

Fig. 4. Dynamics and time of erythrocyte restoration in Bars and Bobka.
The symbols are the same as in Fig. 3.

Time of Restoration of Blood Indices in Experimental and Control Groups

Name of dog	Kind of operation	Restoration time in days	
		hemoglobin	erythrocytes
Moryak	Pancreas denervated	84	40
Laska	The same	66	66
Bars	" "	47	47
Malchik	Ligation of pancreatic ducts	21	27
Mishka	Total pancreatectomy	18	18
Bobka	Total resection of the gland	21	21
Zhuk	Laparotomy	7	7

The pathologico-histological picture of the pancreata after denervation was characterized by the congestion of the small and large veins with blood, their dilatation, and, in one case (the dog Maryak), by a sharp dilatation of the blood and lymph vessels, which suggested the relaxation of their walls. In the intrasecretory apparatus, the islands of Langerhans increased in size and capillary injection and minute grains in the cytoplasm of the cells were observed, which could indicate the hyperfunction of the given apparatus. The latter confirms the clinical data, as we observed temporary hypoglycemia in the clinic. The restoration of the sugar level to normal is probably connected with the supplementary action of compensatory mechanisms.

Therefore, a widespread trauma of the nervous system occurred in our experiments.

Regarding the development process of anemia and taking into account the data we obtained in other experimental series (extirpation of the organ, ligation of the ducts, artificial production of fistulae, alloxan diabetes), we concluded that the changes in erythropoiesis can occur due to the denervation of the pancreas, and not due to the disturbance of some specific hematopoietic function of this organ. Denervation, which disturbs the character of interoceptive impulses, regardless of where the actual operation is performed (carotid sinus, arch of the aorta, spleen, liver) affects the hematopoietic processes for a definite period of time.

The restoration of the normal blood picture after a long time interval perhaps indicates the development of compensating, protective adaptations of the body, which act under the influence of the central nervous system.

SUMMARY

It was shown that denervation of the pancreas in dogs caused anemia lasting from 47 to 84 days, with a 15-20% fall of hemoglobin and 1.8-2.8 million erythrocytes, hyperplasia of the erythroblastic tissue of the bone marrow, and inhibition of the maturation process. In controls (laparotomy, resection of the stomach, ligation of the pancreatic duct or removal of the pancreas) anemia was less pronounced, and of shorter duration. The author is of the opinion that anemia was not caused by some specific effect of the pancreas on the hemopoietic process, but was due to the exclusion of the interoceptive field of the pancreas.

LITERATURE CITED

[1] R. A. Durinyan, Experimental Analysis of Anemias Caused by Denervating Certain Internal Organs,* Avtoreferat Dissertation, Moscow, 1956.

[2] E. L. Kan, Data on the Study of the Nervous System's Influence on Blood Composition,* Avtoreferat Dissertation, Moscow, 1953.

[3] E. L. Kan, Byull. Eksptl. Biol. i Med., 1954, Vol. 37, No. 3, pp. 29-33.

[4] E. L. Kan, Byull. Eksptl. Biol. i Med., 1954, Vol. 38, No. 8, pp. 12-18.

[5] S. G. Kachanova, Byull. Eksptl. Biol. i Med., 1956, No. 1, pp. 11-13.

[6] K. A. Maslovskaya, Byull. Eksptl. Biol. i Med., 1953, Vol. 36, No. 4 (10), pp. 59-63.

[7] O. L. Moiseeva, On the Share of the Liver in the Regulation of Blood Composition,* (Clinical-experimental study), Leningrad, 1953.

[8] O. L. Moiseeva, Byull. Eksptl. Biol. i Med., 1954, Vol. 38, No. 8, pp. 29-32.

[9] N. S. Rozanova and E. A. Zhukova, in the book: Contemporary Problems of Hematology and Blood Transfusion,* Moscow, 1955, p. 85.

[10] A. M. Sarvanov, Change in Blood Composition after Denervation of the Carotid Sinuses and Ligation of the Depressor Nerves,* Avtoreferat Dissertation, Khabarovsk, 1955.

[11] V. N. Chernigovsky and A. Ya. Yaroshevsky, The Problem of the Nervous Regulation of the Blood System,* Moscow, 1953.

[12] A. Ya. Yaroshevsky, Arkh. Patol., 1951, No. 3, pp. 16-20.

* In Russian.

CHANGE IN HIGHER NERVOUS ACTIVITY AND BILE FORMATION IN DOGS WITH EXPERIMENTAL CHOLECYSTITIS

N. K. Gaza

From the Laboratory of Cortico-Visceral Pathology (Head – Prof. L. T. Kurtsin), the I. P. Pavlov Institute of Physiology (Director – Academician K. M. Bykov), Academy of Sciences, USSR, Leningrad

(Received October 11, 1956. Presented by Academician K. M. Bykov)

K. M. Bykov and his colleagues have established that interoceptive signaling affects the function of various internal organs and body systems as well as higher nervous activity. The knowledge of interoception is very important to the explanation of the pathogenesis of diseases in many internal organs, including the liver and gallbladder. As histological studies have shown [8], the gallbladder has an extensive receptor apparatus. Physiological studies have disclosed that when the receptors of a normal gallbladder are mechanically stimulated, the higher nervous activity of animals changes [2,5,6,7]. When mechanical, thermal or chemical factors act upon the mucous membrane of the stomach or intestine, the secretion and motoricity of these organs changes [1,3,-9,10].

Disturbances of higher nervous activity were obtained in experimental gastritis and stomach ulcers [4].

We studied the condition of the cerebral cortex in dogs with experimental cholecystitis and the functional condition of the gallbladder mechanorecceotors before and after cholecystitis.

EXPERIMENTAL METHODS

The experiments were done on two dogs with a developed and fixed stereotype of exteroreceptive and interoreceptive conditioned reflexes.

The dogs had fistula of the gallbladder with a ligated common bile duct. The higher nervous activity in the dog Sekret was classified as the weak type, that in the dog Alfa, as a strong variation of the weak type.

We determined the functional condition of the gallbladder mechanoreceptors by the changes in the higher nervous activity during mechanical action on the gallbladder. A rubber balloon was inserted into the gallbladder cavity through the fistula (size of ballon: 4 x 2 cm). After the start of the experiment, 25 ml of water with a temperature of 35° was introduced into the ballon. The balloon was left filled in one dog until the end of the conditioned reflex experiment, but, in the other, only during the action of the first two conditioned stimuli, from then on the interoceptive, conditioned reflex from the gallbladder followed. Inflammation of the gallbladder (cholecystitis) was produced by one or two irrigations of its mucous membrane with a 5% solution of silver nitrate.

EXPERIMENTAL RESULTS

The first series of experiments – stimulation of the gallbladder mechanoreceptors – was begun on a background of normal higher nervous activity in the animals. When the gallbladder mechanoreceptors were stimulated, a change occurred in the higher nervous activity, mainly expressed by a decrease in the potency of the conditioned reflexes. In many experiments, one could observe the appearance of the regular and paradoxical phases in the activity of the grey matter cells and the disinhibition of the differentiation. The unconditioned reflexes were essentially unchanged. In the intervals between the experiments with bladder stimulation, higher nervous activity in the dogs returned to the original level.

Fig. 1. Changes in higher nervous activity and bile secretion under the influence of stimulation of the gallbladder mechanoreceptors in Alfa.
1) result of positive conditioned reflexes; 2) bile secretion to meat (100 g). The space between the arrows shows the experiments stimulating the gallbladder mechanoreceptors.

The changes in the bile formation function of the liver in the dog during the experiments with gallbladder mechanoreceptor stimulation were characterized by fluctuations in the amount of bile secreted in response to food stimuli (Fig. 1).

The data we obtained agree with those of other authors who studied the influence of gallbladder receptor stimulation on higher nervous activity [2,5,7].

The second series of experiments – inflammation of the gallbladder – was also begun with normal higher nervous activity in the animals.

After one irrigation of the gallbladder mucous membrane with the 5% silver nitrate solution (15 ml), a long disturbance of the higher nervous activity occurred in the dog Sekret, and it was especially strongly expressed the first month after the irrigation. The higher nervous activity disturbances were expressed by a decrease in the result of the positive conditioned reflexes to 2-6 drops instead of the original 9-12. The conditioned reflexes remained weakly expressed for 5 weeks. During that period, the regular and paradoxical phases in the activity of the cerebral cortex cells were clearly evident. In many experiments, the differentiation was disinhibited and exceeded the potency of the positive conditioned reflex. The animal refused to eat in almost every experiment. The product of the unconditioned reflexes was reduced to 30 drops of saliva in some experiments instead of the initial 120-130 drops. Along with the bile, a serous-purulent discharge was excreted from the gallbladder. The bile secretion decreased during food ingestion; for example, 25 ml of bile (average of 13 experiments) was secreted to meat in 4 hours instead of the normal 30 ml. After 5 weeks, the dog was given $1\frac{1}{2}$ months rest, after which it no longer refused food during the experiments. The higher nervous activity began to be gradually restored and finally, after 5 months, returned to the original level.

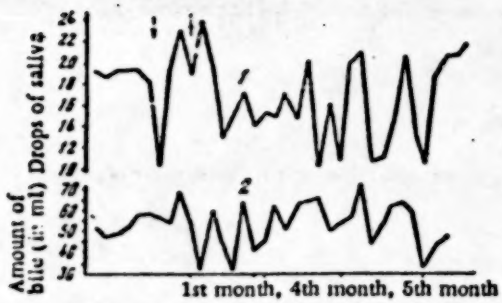

Fig. 2. Changes in higher nervous activity and bile excretion during experimental cholecystitis in Alpha. The symbols are the same as in Fig. 1. The arrows show the experiments with irrigation of the gallbladder mucous membrane with a 5% solution of silver nitrate.

In Alfa, the first irrigation of the gallbladder mucous membrane with the 5% silver nitrate solution first caused the degree of the conditioned reflexes to decrease (their product decreased from 18-19 drops to 10), but then caused it to increase (to 23 drops). Two weeks after the first irrigation, a second was done. It first caused a temporary increase, then a decrease of the conditioned reflexes (to 13-15 drops); the regular and paradoxical phases were observed in the activity of the cerebral cortex cells. The unconditioned saliva-secreting reflexes were essentially unchanged. Disturbances of higher nervous activity were also observed after the $1\frac{1}{2}$ month interruption in the work, and lasted on the whole for a five month period (Fig. 2, 1).

Changes in the bile forming function of the liver, during gallbladder inflammation, were expressed by sharp fluctuations in the amount of bile secreted to food stimuli. For example, 35-70 ml of bile were secreted to 100 g of meat for 4 hours of the experiment instead of the original 45-55 ml (Fig. 2, 2). The bile was cloudy, with a large quantity of mucus and streaks of blood.

This data indicates that impulses leaving the pathologically altered organ change the higher nervous

activity and that, possibly in connection with this, the functions of the various organs are altered. The secretory function of the liver particularly changes, as our studies showed.

Gallbladder motoricity during cholecystitis was also studied. The contractions were recorded by a water-air transmission and a Marey's capsule on a kymograph tape before and after the food stimulus had been given (2 egg yolks). While the inflammation was developing in the gallbladder, the motor activity of the gallbladder was disturbed in both dogs (Fig. 3). The rhythmic contractions of the gallbladder lessened considerably during the period of inflammatory phenomena in the bladder. This was evident even before the food stimulus had been given, but especially after ingestion of the two egg yolks.

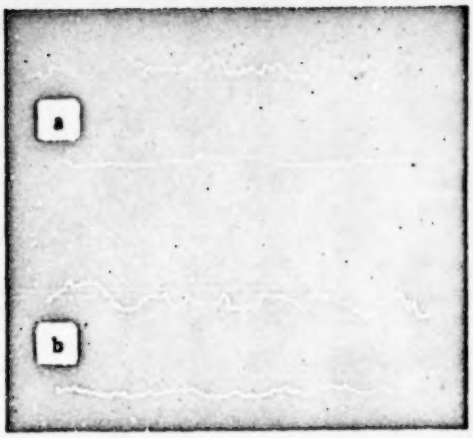

Fig. 3. Change in gallbladder motoricity during experimental cholecystitis in Sekret (a) and in Alfa (b).
1) motoricity of the bladder 10 minutes after ingestion of 2 egg yolks, before cholecystitis; 2) the same with cholecystitis.

In a third series of experiments, we examined gallbladder reception after cholecystitis. It could be proposed that inflammation decreases the sensitivity of the gallbladder receptors. However, the experiments showed that the reverse was true. In Sekret, a second stimulation of the gallbladder mechanoreceptors by the same stimulus as was used in the first series (25 ml of water) caused change in the higher nervous activity. The degree of the conditioned reflexes decreased, and the differentiation was disinhibited.

In Alfa, even after 5 months, the potency of the conditioned reflexes and the degree of bile formation was unstable. The bile secreted contained much mucus. Stimulating the bladder mechanoreceptors by inflating the balloon caused, at that time, more agitation of the animal than in the first series of experiments and vomiting motions. From the standpoint of the higher nervous activity, a sharp suppression of the positive conditioned reflexes was observed (in one experiment, they disappeared).

Therefore, with experimental cholecystitis, we observed that higher nervous activity was suppressed for a protracted period and that the bile-forming function of the liver was also disturbed. Five months after the irrigation of the gallbladder mucous membrane with the 5% solution of silver nitrate, the sensitivity of the gallbladder receptors was slightly increased.

SUMMARY

Two dogs with fistulae of the gallbladder and established stereotypes of salivary conditioned reflexes were studied. Observations demonstrated that in case of cholecystitis caused by one or two irrigations of the mucous membrane of the gallbladder with a 5% solution of silver nitrate, positive conditioned reflexes were lowered, differentiation was disinhibited for a long time (several months), bile formation in the liver was decreased, and the motor activity of the gallbladder was lowered.

LITERATURE CITED

[1] K. M. Bykov and I. T. Kurtsin, The Corticovisceral Theory of the Pathogenesis of Ulcerous Diseases," Moscow, 1952.

[2] Ya. V. Ganitkevich, Fiziol. Zhur. SSSR, 1955, Vol. 1, No. 4, pp. 5-14.

[3] I. T. Kurtsin, Fiziol. Zhur ., SSSR, 1938, Vol. 25, No. 6, pp. 885-905.

[4] I. T. Kurtsin, A. New Method of Functional Diagnosis for the Diseases of the Human Stomach," Moscow, 1953.

[5] A. M. Nikitina, Summary of the Reports of the 9th Conference on Physiological Problems,* Moscow-Leningrad, 1941, pp. 59-60.

[6] A. M. Nikitina, Uchenge Zapiski Leningrad. Gosudarst. Pedagog. Inst. im. 1947, Vol. 60, No. 3, pp. 79-142.

[7] V. G. Prokopenko, The Cerebral Cortex and the Secretory Activity of the Liver,* Dissertation, VIEM Leningrad, 1939.

[8] M. K. Rodionov, in the book: Morphological Questions Regarding the Receptors of the Internal Organs and the Cardiovascular System,* Moscow-Leningrad, 1953, pp. 62-75.

[9] S. I. Frankshtein, The Reflexes of Pathologically Altered Organs,* Moscow, 1951.

[10] O. G. Chumburidze, cited by I. T. Kurtsin, A New Method of Functional Diagnosis for the Diseases of the Human Stomach,* Moscow, 1953, p. 53.

* In Russian.

THE EFFECT OF HIGHER NERVOUS ACTIVITY DISTURBANCE ON HEMOGLOBIN METABOLISM

T. V. Fokina

From the Pathophysiological Laboratory (Head – Prof. N. M. Nikolaev), the Institute of Pediatrics (Director – O. D. Sokolova-Ponomareva, Corresponding Member of the Academy of Medical Sciences, USSR), the Academy of Medical Sciences, USSR, Moscow

(Received June 7, 1956, Presented by Academician G. N. Speransky)

The influence of the nervous system on hemoglobin metabolism has not yet been sufficiently explored. The data in the literature regarding the influence of the nervous system on the peripheral blood picture [1,2,3-7, 9, 10], in most cases, refers only to leukocytes. There is much less work regarding the influence of the nervous system on the red blood [1, 4, 5, 12] and in the few existing works, the authors barely mention the influence of the nervous system on hemoglobin decomposition intensity. As is known from the works of N. M. Nikolaev [8], G. F. Lang [6], Ya. G. Uzhansky [11] and others, there is a very close relationship between the processes of erythrocyte hemoglobin regeneration and decomposition. Therefore, in studying red blood changes, one must examine the indices of both the intensity of the hemolytic processes and the intensity of erythrocyte hemoglobin regeneration.

The purpose of this work was to study the effect of disturbance in higher nervous activity on the decomposition and synthesis of hemoglobin experimentally, in order to more nearly approach the explanation of the possible parts played by the nervous system in the pathogenesis of various anemias.

EXPERIMENTAL METHODS

To study hemoglobin metabolism, the intensity of hemoglobin decomposition was determined from the stercobilin excreted with the stool (by Terven's method) and the intensity of erythrocyte regeneration was determined from the amount of reticulocytes in the blood, the level of hemoglobin and the number of erythrocytes.

The experiments were done on 4 dogs; higher nervous activity was disturbed in 2 of the dogs by combining the food and defense unconditioned reflexes, and in the other 2 by combining the inhibitory and stimulatory processes after first producing conditioned defense, electric skin reflexes to the continuous sound of a bell and a differentiation to its interrupted ringing. A total of 16 experiments were done combining the unconditioned reflexes, and 62 were conducted with the second variant of the method.

During the experiments, daily stercobilin excretion was determined before during and after the combination. Examination of stercobilin content in the stool was done on a two-day amount of stool. A total of 124 stool analyses for stercobilin excretion were done on the 4 dogs. Blood was taken periodically from the ear before and many times during the experiment (44 blood analyses were done).

EXPERIMENTAL RESULTS

In the dog Belka, weighing 7.8 kg, higher nervous activity was disturbed by combining the food with

defense unconditioned reflexes. The dog was put in a chamber and given a trough of meat; then, the moment the dog touched the meat, his paw was given an electric shock. After the first combination of the food with the painful stimulation, the dog no longer touched the meat, but turned away and afterwards, whenever he saw the meat, began to yelp and tear away from the straps. It was very difficult to lead the dog into the chamber; it resisted, snapped and whined, but, nevertheless, the combination was produced daily.

Stercobilin excretion before the collision was from 1.9 to 2.7 mg per day. After the collision, stercobilin excretion increased and reached the maximal level (7.6 mg) about the 3rd-5th day, then decreased to 3.4 mg per day. The amount of reticulocytes increased from 3.5 to 6.5%, but the hemoglobin level decreased slightly – from 18.3 to 17.9 g%.

A combination was produced by the same means as in Belka in the dog Palma, which weighed 11.4 kg. After the very first combination of the food and defense unconditioned reflexes, the dog became very aggressive, pulled away from the straps and yelped. In this dog, stercobilin excretion was from 1.1 to 9.2 mg per day before the combination, but, by the 6th day of the experiment, it increased to 36.7 mg, i.e., was almost 4 times greater than before the combination. On the 7th-8th day, stercobilin excretion decreased to 13.1 mg per day. The amount of reticulocytes did not change. By the 6th day, the hemoglobin level had decreased from 19.8 to 17.9 g% (Fig. 1).

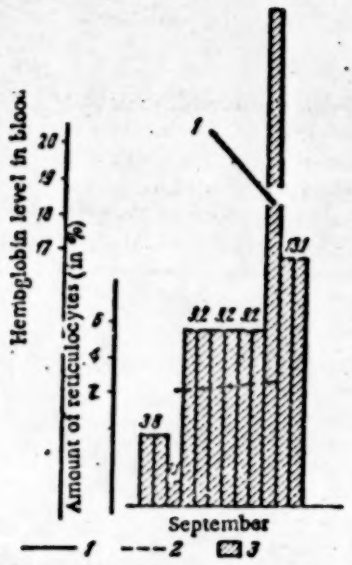

Fig. 1. Effect on hemoglobin metabolism in the dog Palma of combining the food and defense reflexes.
1) hemoglobin level in blood; 2) amount of reticulocytes; 3) stercobilin excretion in mg per day.

Therefore, when food and defense unconditioned reflexes were combined a temporary intensification of hemoglobin decomposition was observed: pigment excretion in the stool increased, and the level of hemoglobin slightly decreased.

In the dog Sharik, (6.8 kg) higher nervous activity was disturbed by colliding the stimulatory and inhibitory processes. A positive conditioned reflex to a continuous sound with an electric skin reinforcement was first produced in the animal. The answering reaction was expressed by the paw being jerked back, which reaction was kymographically recorded. A differentiation was produced to a bell which rang with interruptions (60 interruptions per minute). The action time of the conditioned stimulus was 20 seconds, and the time of the combined action of the conditioned and unconditioned was 5 seconds. The conditioned reflex developed slowly in this dog. It appeared first at the 8th combination, but was not permanent until the 63rd. The latent period of the conditioned reflex was from 2 to 8 seconds. The conditioned reflex was clearly expressed. We began to produce the differentiation in the dog after the 22nd experiment, and this appeared after the very first combination. During the development of the differentiation, the dog fell from time to time into an inhibited condition, and the conditioned reflex, at first, often disappeared.

When the experiment with the combination was begun, the conditioned reflex was firmly established in the dog, and the differentiation was absolute.

In the experiment with the combination, we gave the continuous bell sound for 20 seconds, then, immediately after this bell, we rang the interrupted bell for 20 seconds, then again the continuously ringing bell, and then the electric current reinforcement. Gradually the number of alternating conditioned stimuli was increased to 5, 7, 9 up to 25. At first, the experiments were done every day, then with small interruptions. The activity of the nervous processes in the animal was good; the dog reacted quickly to the continuous bell sound by jerking back its paw, did not move its paw to the interrupted bell, then jerked it back again to the continuous

bell sound. After frequent repetition of such experiments, it became very difficult to lead the dog into the chamber and tie it. It tore away, snarled and even bit. In the chamber, it yelped and ran about, but during the experiment worked very efficiently. By the 2nd month of the combination experiments, the dog was so much more aggressive that the experiments were sometimes disrupted, since sometimes the dog could not be gotten into the chamber.

In the first month of the combination experiment, no substantial disturbance in hemoglobin metabolism was evident, either in stercobilin excretion or in hemoglobin level. Only some fluctuation in the amount of reticulocytes was observed (sometimes increased to 9-10%). After the second month, hemoglobin decomposition became intensified, and stercobilin excretion often increased to 14.9, 15.8, or even to 29.1 mg per day (maximum before experiment was 10 mg). During this period, the amount of reticulocytes sometimes increased to 13, 18 and 19%. At first, intensified erythrocyte regeneration compensated for the increased rate of hemoglobin decomposition, and the hemoglobin level remained the same. Only 2½ months after the combinations were started, when there were as many as 25 alternations of the positive and inhibitory stimuli did stercobilin excretion increase to 29.1 mg, the hemoglobin level fall from 19.3 to 16.2 g%, and the amount of reticulocytes increase g more steadily to 10.5-18%. Then the combination was stopped, and after 2-3 weeks, pigment excretion decreased, and the hemoglobin level increased gradually until it reached the original level. However, the amount of reticulocytes remained high (8.5-10%) (Fig. 2).

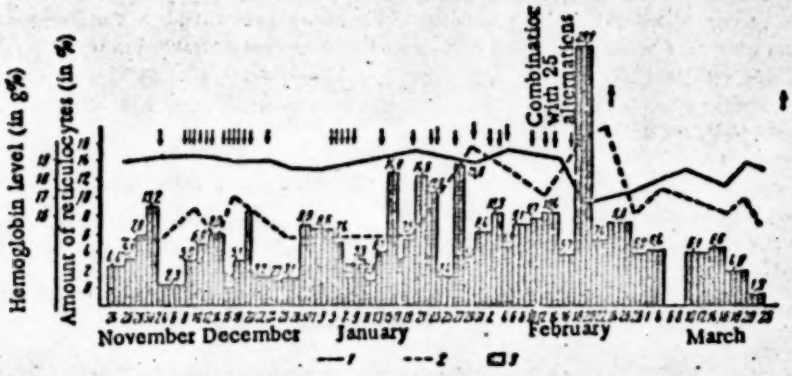

Fig. 2. Effect of combining the stimulatory and inhibitory processes on hemoglobin metabolism in the dog Sharik.
1) level of blood hemoglobin; 2) amount of blood reticulocytes; 3) stercobilin excretion in mg per day; arrows indicate the interruption in the work with conditioned reflexes.

A defense conditioned reflex was also produced in the dog Snezhka (5.3 kg) to a continuous bell sound and a differentiation to an interrupted bell sound. The conditioned reflex developed quickly, first appearing at the 5th combination. The latent period varied, from 1 to 10 seconds. The differentiation production was started early in this dog, when the conditioned reflex was not yet completely permanent (after 12 combinations in the development of the conditioned reflex). The differentiation developed very quickly, first appearing at the 2nd combination, and becoming absolute in almost all experiments after the 5th combination. The collision was produced in the same way as in the dog Sharik. The dog began to become aggressive as a result of the combination experiments.

Before the combination, the hemoglobin level was 18.3 to 19.7 g%, the number of reticulocytes 2 to 4 % and stercobilin excretion 3.5 to 5.8 mg per day. After the beginning of the combination experiments the hemoglobin level remained unchanged for a month. Stercobilin excretion increased the first days after the start of the experiment and, for the first week, was 7.8 to 10.5 mg. Later, from the 2nd to 5th weeks, periodic elevations in stercobilin excretion to 13.5-14.9 mg per day were observed in the course of its generally intensified excretion.

After the intensification of hemoglobin decomposition (stercobilin excretion was used as the criterion), an increase in the amount of reticulocytes was observed, to 8% on the 10th day and 10.5% on the 15th day, which indicated the activation of erythropoiesis. Due to the latter, the hemoglobin level remained steady for the first month.

But, 1½ months after the beginning of the combination, the hemoglobin level decreased to 1.5 g% of the original and continued to decrease, after a temporary increase to the normal level on the 56th day, reaching the minimum (3 g% lower than the original) after 2½ months. After the experiments were stopped, the hemoglobin level began to increase gradually.

The degree of fluctuation in the number of reticulocytes during the final 1½ months of the combination experiments was between 2.5 to 9.5%. One must point out that hemoglobin metabolism was observed to be unstable even some time after the combination experiments had ceased.

The last two experiments showed that an instability in hemoglobin metabolism appeared, with a predominance of the hemoglobin decomposition processes, due to the experiments colliding higher nervous activity in dogs.

The data presented show that the activity of the hemolytopoietic system depends greatly on the functional condition of the cerebral cortex and that the regulation of hemoglobin synthesis and decomposition is disturbed during the development of a neurotic condition. Hemoglobin decomposition at first increases, then erythrocyte regeneration is intensified, which causes the hemoglobin level of the blood to remain essentially the same for a certain period of time. Aggravation of the neurotic state leads to the disruption of this compensation, after which the hemoglobin level of the blood becomes considerably lower.

SUMMARY

The effect of higher nervous activity disturbances on hemoglobin metabolism in the dog was studied. The disturbances were caused by the combination of food and defense unconditioned reflexes and by a combination of inhibitory and excitatory processes, after a conditioned defense electric skin reflex and its differentiation had been previously formed. It was discovered that after the development of the neurotic state caused by such disturbances, hemoglobin decomposition in animals was intensified. Due to the compensatory augmented erythrocyte formation, the hemoglobin level of the blood did not materially change at the beginning, but with the progressive aggravation of the neurotic state, compensation failed and the hemoglobin level considerably decreased.

LITERATURE CITED

[1] B. I. Banduyarov, The Trophic Function of the Brain,* Moscow, 1948.

[2] V. A. Beier, 1950, Vol. 28, No. 9, pp. 45-51.**

[3] G. S. Belenky, Med., 1950, Vol. 28, No. 9, p. 52.

[4] N. S. Dzhavadyan, Klin. Med., 1951, No. 9, pp. 71-74.

[5] T. S. Istamanova, Arkh. Biol. Nauk SSSR, 1936, Vol. 42, No. 2, pp. 43-47.

[6] G. F. Lang, Klin. Med., 1939, Vol. 17, No. 6, pp. 3-13.

[7] P. Ya. Mytnik, Arkh. Biol. Nauk, SSSR, 1937, Vol. 45, No. 1, pp. 133-150.

[8] A. M. Nikolaev, Byull. Eksptl. Biol. i Med., 1929, Vol. 2, No. 29, pp. 67-77.

[9] N. M. Nikolaev, Klin. Med., 1954, Vol. 32, No. 2, pp. 31-37.

[10] L. A. Trubnikova, Arkh. Patol., 1955, Vol. 17, No. 1, pp. 63-64.

[11] Ya. G. Uzhansky, The Role of Erythrocyte Destruction in the Mechanism of Blood Regeneration,* Leningrad, 1949.

* In Russian.

** Journal title omitted – Publisher.

ANALYSIS OF INTEROCEPTIVE REFLEXES IN EXPERIMENTAL TUBERCULOSIS

Article III

THE EFFECT OF TUBERCULIN UPON REFLEXES FROM THE CHEMORECEPTORS OF AN ISOLATED LOOP OF THE SMALL INTESTINE IN HEALTHY CATS

V. A. Strelets

From the Department of Experimental Pathology and Therapy (Head – Candidate of Medical Sciences, G. S. Kan), Leningrad Institute for Tuberculosis Research (Director – Prof. A. D. Semenov, Research Consultant, V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

(Received June 10, 1956. Presented by V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

In works published earlier, we presented data regarding the effect of tuberculin on reflexes from carotid sinus receptors and from the mechanoreceptors of the internal gonads [1,2]. We established that the reflexes from the carotid sinus receptors on the blood pressure were more sensitive to the toxic effect of tuberculin on animal bodies than were the reflexes from the mechanoreceptors of the gonads. We suggested that this difference was caused by the fact that both the baroreceptors of the carotid sinus and the chemoreceptors of the carotid plexus participated in the reaction to pressure on the carotid artery.

The purpose of the present work was to prove the hypothesis that the reflexes from the chemoreceptors are less resistant to the toxic effect of tuberculin than are the more stable reflexes from the mechanoreceptors.

EXPERIMENTAL METHODS

The method we used consisted of perfusing an isolated loop of the small intestine. The experiments were done on cats under urethane anesthesia.

After the celiac cavity had been opened, the small intestine was freed, and a loop of it near Bauhin's valve 20-25 cm long was isolated from the rest of the intestine, immersed in a vessel with Tyrode's nutritive solution and heated to a temperature of 37°. A glass tube was inserted into the distal end of the isolated intestinal loop in order to remove the intestinal contents. Then the neurovascular plexus was freed from the surrounding tissues. The nerves entwining the artery were carefully freed, and the vein and artery ligated. All the surrounding tissues, including the mesenteric glands, were ligated. A cannula was inserted into the artery in order to introduce the previously heated, oxygenated Tyrode's solution. The solution was withdrawn through a cannula inserted into the vein. Therefore, the only way in which the isolated intestinal loop was still connected with the body was through the nerves.

The condition of reflex excitability of the chemoreceptors in the perfused portion of the intestine was determined by the blood pressure and respiration reactions which were effected by adding to the Tyrode's solution 1 ml of a nicotine solution (10^{-5}) or 1 ml of an acetylcholine solution (10^{-2}, 10^{-4}), and also by the temporary replacement of the oxygenated solution with a solution containing carbon dioxide.

The blood pressure in the carotid artery was recorded with a mercury manometer, and the respiration, by means of a Marey capsule, which was connected to the animal's trachea by a glass T-tube.

After a background of the original blood pressure and respiratory reflex reactions caused by the introduction of acetylcholine, nicotine or carbon dioxide into the intestinal perfusion current had been established, the animal was intramuscularly injected with tuberculin. Old Koch's tuberculin was injected in a dose of 4-10 ml

– 8-12 mg of a purified dry preparation diluted with 8-12 ml respectively of a physiological solution. A total of 18 experiments were done. In three experiments, the reflex reactions from the chemoreceptors in response to the three kinds of stimulants – acetylcholine, nicotine and carbon dioxide – were examined. In five experiments, they were activated by two stimulants – acetylcholine and nicotine or acetylcholine and carbon dioxide. In the other ten experiments, either acetylcholine alone or carbon dioxide alone were used as the stimulants. The reflexes from the bladder mechanoreceptors were also examined in three experiments. Such composite experiments made it possible to compare the effect of tuberculin on the reflex excitability of the basomotor and respiratory centers with the stimulants coming from different kinds of receptors under the same experimental conditions.

EXPERIMENTAL RESULTS

After the tuberculin injection, in 17 experiments the degree of the reflexes from the chemoreceptors on the blood pressure either decreased (by 14.2-87.5%), or the reflexes totally disappeared (5 experiments). Only in one experiment using carbon dioxide as the stimulant did the reflex on the blood pressure increase after the tuberculin injection by 22.2%.

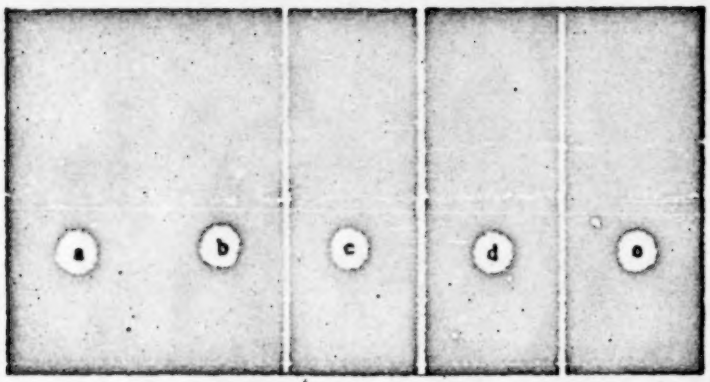

Fig. 1. Inhibition of reflex from small intestine chemoreceptors and sharp decline of average level of blood pressure after intramuscular injection of 9 ml of tuberculin, with the introduction of a 10^{-4} solution of acetylcholine.
a,b) original reflex; c,d,e) reflex after 10 minutes, 30 minutes and 1 hour following the injection of tuberculin. The curves from top to bottom signify: blood pressure, respiration, indication of stimulation, indication of time (in 5 second marks).

The decrease or disappearance of the reflexes most frequently occurred during the first 10-30 minutes after the tuberculin injection (in 14 out of 17 experiments). In 9 experiments, no greater inhibition of the reflexes from the chemoreceptors than that which occurred during the first 10-30 minutes was observed. The reflex either remained decreased to almost the same degree for the 1-1½ hour observation period after the tuberculin injection (Fig. 1) or increased, sometimes even reaching the original level. In 5 experiments out of 14, the reflexes which had decreased during the first 10-30 minutes after the tuberculin injection showed an even greater tendency to decrease during the subsequent observation (Fig. 2).

In 3 experiments out of 17, the reflexes from the chemoreceptors only became inhibited an hour after the tuberculin injection. Up to this time, the reflexes either remained the same as before the tuberculin injection or even slightly increased.

The arterial pressure level did not change much in the majority of experiments. A sharp decline of the average blood pressure was observed shortly after the tuberculin injection in only 2 experiments – 45% in both cases (see Fig. 1).

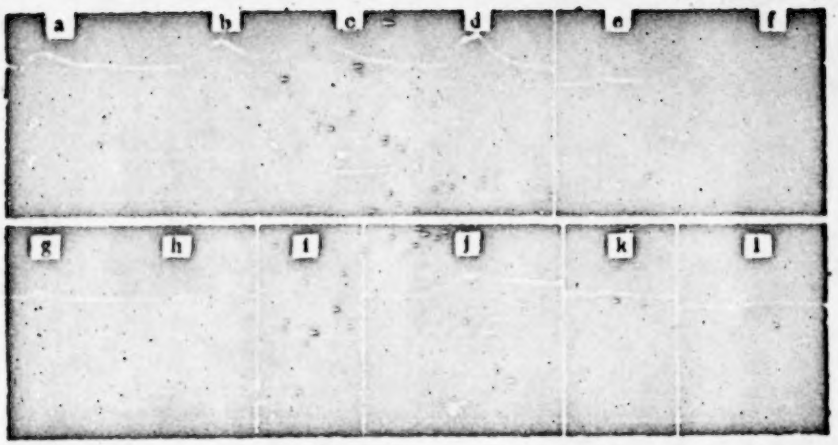

Fig. 2. Decrease in reflexes from the small intestine chemoreceptors after intramuscular injection of 4 ml of tuberculin.
a,b,c,d) original reflexes; e,f,g,h,i,j,k,l) reflexes after tuberculin injection; 1) with introduction of 10^{-4} acetylcholine; 2) with introduction of CO_2. The Curves are the same as in Fig. 1.

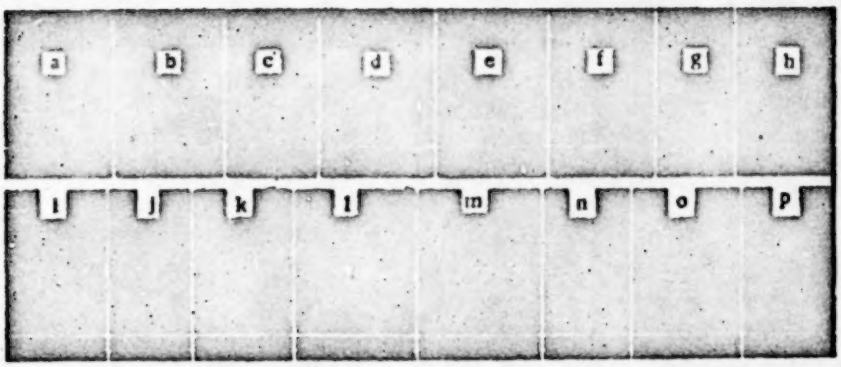

Fig. 3. Decrease of reflexes from small intestine chemoreceptors and increase of reflexes from bladder mechanoreceptors after injection of 5 ml of tuberculin.
a,b,c,d) original reflexes; f,g,h,k,l,n,o,p) reflexes from chemoreceptors after tuberculin injection; e,i,m) reflexes from mechanoreceptors after tuberculin injection. 1) reflexes from bladder when it was inflated under pressure of 90 mm of mercury; 2) with introduction of 10^{-4} acetylcholine; 3) with introduction of 10^{-5} nicotine; 4) with introduction of CO_2. The curves are, from top to bottom: blood pressure, respiration, indication of time (in 5 second marks), indication of stimulation.

In all of the experiments, the respiratory reflex reactions were either unchanged or very slightly inhibited.

The reflexes from the bladder mechanoreceptors increased in all 3 experiments in which they were examined after the tuberculin injection (Fig. 3) in contrast to the reflexes from the chemoreceptors.

The control experiments showed that the reflexes from the chemoreceptors, in 2 hours of observation, did not show such considerable oscillations as appeared after the tuberculin injection.

DISCUSSION OF RESULTS

The observations made from our first experimental series showed that tuberculin is far from inert in relation to the interoceptive reflexes. The most characteristic effect of tuberculin was that it first inhibited the reflexes from the chemoreceptors. This appeared most distinctly in the perfusion experiments on the intestine, as well as in the experiments with the carotid body reflex, which, with our method of stimulation, depended on stimulation of both the baroreceptors and the chemoreceptors. The effect of tuberculin on the reflexes from the bladder mechanoreceptors was much less inhibitory. This fact, we believe, indicates that tuberculin has an inhibiting effect on those central nervous elements in the afferent part of the reflex arc which are connected with the tissue chemoreceptors. This effect could not have been produced by the direct action of tuberculin on the chemoreceptors, as we know to be possible from the experiments of E. A. Levtova [3]. In our experiments, the reflex from the chemoreceptors was reproduced from the perfused loop of the small intestine, which had no humoral relations with the body, and was therefore not subject to the direct influence of the preparation.

The effect described was evidently not connected with a direct inhibitory effect of tuberculin on the efferent part of the reflex arc (vasomotor center and connection of this with the heart and vessels) nor on the effector mechanisms (heart and vessels). The absence of such an effect was especially evident when, under the same experimental conditions, the reflexes from the chemoreceptors were compared with those from the mechanoreceptors, which comparison showed that the former were inhibited considerably by tuberculin, while the latter not only were not inhibited, but often even increased. Therefore, while the impulses from the chemoreceptors to the effector organs (heart and vessels) became blocked, the impulses from the mechanoreceptors proceeded freely.

Therefore, tuberculin, penetrating the brain, acts electively in the subcortical region on the central nervous elements entering the afferent route from the tissue chemoreceptors. We propose that this effect is not realized in the cerebral cortex, since the experiments were conducted under anesthesia.

The inhibitory effect of tuberculin on the reflexes from the chemoreceptors appeared with the use of doses far greater than those used for diagnostic purposes in people and animals. Therefore, one could assume that it was simply a question of the toxic effect of the preparation. This must be clarified in the event of possible misunderstandings. In the control experiments, conducted on 4 cats, it was shown that the doses we used in the experiments studying interoceptive reflexes (3-10 ml) could not be considered vitally toxic to the experimental animals. The intramuscular injection of as much as 25 ml of tuberculin into the cats, with or without anesthesia, did not cause death in the experimental animals either during the first hours of observation or during subsequent days. It may only be a question of the elective toxicity typical to any poison or pharmacological substance. If, in the dose we used, tuberculin had had simply a toxic effect on the body, then the reflexes would have disappeared from the mechanoreceptors as well as from the carotid sinus receptors. This, however, was not the case in our experiments. Therefore, the inhibitory effect of tuberculin on the reflexes from the chemoreceptors is a manifestation of the elective toxicity inherent in the subject stimulant.

SUMMARY

It was shown that in its common effect upon healthy cats, tuberculin first of all inhibits reflexes arising from the chemoreceptors. This effect was most marked in perfusion experiments on the intestine, as well as in experiments with the carotid sinus reflex. Inhibition of reflexes from mechanoreceptors of the urinary bladder was less marked. The assumption is made that in its general effect, tuberculin inhibits the central neural elements of the afferent section of the reflex arc associated with tissue chemoreceptors.

LITERATURE CITED

[1] V. A. Strelets, Byull. Eksptl. Biol. i Med., 1953, Vol. 36, No. 9, pp. 22-25.

[2] V. A. Strelets, Byull. Eksptl. Biol. i Med., 1953, Vol. 36, No. 12, pp. 34-37.

[3] P. A. Levtova, Byull. Eksptl. Biol. i Med., 1952, No. 7, pp. 35-39.

THE EFFECT OF PNEUMOPERITONEUM ON ASEPTIC LEUKOCYTOSIS CAUSED BY PARENTERAL INJECTION OF MILK

K. A. Pimenova

From the Experimental Section (Head – G. S. Kan, Candidate for Medical Sciences), Leningrad Institute of Tuberculosis (Directors – Prof. A. D. Semenov, Research Consultant; V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR)

(Received October 30, 1956. Presented by V. N. Chernigovsky, Active Member of the Academy of Medical Sciences, USSR

In earlier published works [6,7], we showed that the inducement of pneumoperitoneum in animals produced marked changes in the excitability of the basomotor center, thermoregulation and the respiratory center.

The purpose of this work was to study the effect of pneumoperitoneum on leukocytosis caused by the intramuscular injection of boiled skim milk.

EXPERIMENTAL METHODS

When the mechanoreceptors and chemoreceptors were stimulated, changes appeared in the composition and quantity of the peripheral blood leukocytes [1,2,3,8]. The part of the reflex component in these leukocyte reactions has been proved [4,5,9,10,11].

In our experiments, the leukocyte reaction was caused by intramuscularly injecting cats with 3 ml of boiled skim milk, under aseptic conditions.

The studies were done on 8 male cats. 39 experiments and 248 hematological examinations were done in all. Each animal was examined every 15-30 days. The milk was injected into a different paw in each different experiment.

The observations were so conducted that the control studies with the milk injection (without the injection of air into the celiac cavity) alternated in each animal with the experiments, in which pneumoperitoneum had been preliminarily induced. Besides, the spontaneous fluctuations occurring in the peripheral blood leukocyte content under no influence other than that of the experimental set-up were examined in each animal over a period of 4 hours.

The number of leukocytes in the peripheral blood was determined before the milk injection and then, 30 minutes, 1,2,3, and 4 hours after the injection. Blood was taken from an incision made in the ear with sharp scissors.

In the experiments, small volumes of air were injected into the celiac cavity in 9 cases (100-150 ml), and large volumes in 3 cases (300-400 ml). Pneumoperitoneum was induced 1-1½ hours before the milk injection. During this period, no appreciable changes in the number of leukocytes in the peripheral blood appeared.

EXPERIMENTAL RESULTS

Leukocytosis, in accordance with the data in the literature, developed in the control observations with the intramuscular milk injection. The temporary and slight decrease in the original number of peripheral blood leukocytes, which occurred right after the milk injection, usually became, within an hour or two, acute leukocytosis, which continued for the next two hours of observation.

In the experiments in which pneumoperitoneum had been induced, leukocytosis also developed before the milk injection, but the features of the leukocyte reaction effected by the pneumoperitoneum were different.

TABLE 1

The Effect of Pneumoperitoneum (small doses) on Leukocytosis Caused by the Parenteral Injection of Skim Milk (average of all experiments)

Time interval in hours after milk injection	Numerical increase of leukocytes in control experiments (in %)	Numerical increase of leukocytes in experiments with pneumoperitoneum (in %)	Difference in leukocyte numerical increase in experiments with and without pneumoperitoneum (in %)
2	108	91	—17
3	167	94	—73
4	181	128	—53
Average	152	104	—48

TABLE 2

Effect of Pneumoperitoneum (large doses) on Leukocytosis Caused by Parenteral Injection of Milk

Time interval after milk injection (in hours)	Numerical increase of leukocytes in control experiments (in %)	Numerical increase of leukocytes in the experiments with pneumoperitoneum (in %)	Difference in numerical increase in experiments with and without pneumoperitoneum (in %)
2	102	130	+28
3	158	160	+ 2
4	172	186	+14
Average	144	159	+15

In 8 out of 9 experiments in which a small dose of air (100-150 ml) had been preliminarily injected into the celiac cavity, the leukocyte reaction to the parenteral injection of milk was less marked than in the control (Fig. 1).

The change in the number of leukocytes in the experiments and in the control is shown in Table 1.

Table 1 shows that the average numerical increase of leukocytes 2, 3 and 4 hours after the milk injection (the results were not determined during the first hour due to the fact that the original number of leukocytes usually decreased considerably during that period) was 48% less in the experiments than in the control.

Therefore, the preliminary injection of small volumes of air into the celiac cavity causes the leukocyte reaction, developing due to the milk injection, to be moderated, and decreases the intensity of the developing leukocytosis.

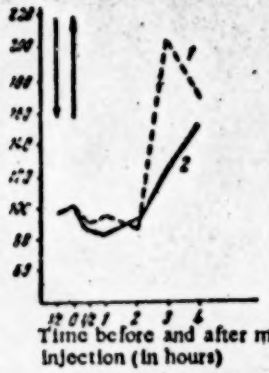

Fig. 1. Effect of pneumoperitoneum caused by the injection of a small volume of air (125 ml) on leukocytosis caused by the intramuscular injection of milk.
1) control; 2) experiment; ↓ – air injection; ↑ – milk injection.

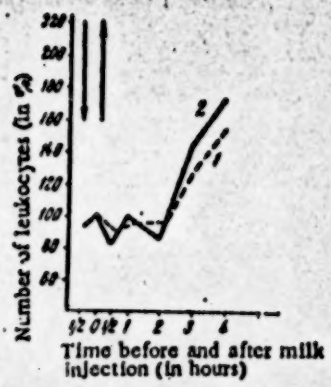

Fig. 2. Effect of pneumoperitoneum caused by injection of large volume of air (375 ml) on leukocytosis caused by intramuscular injection of milk. The symbols are the the same as in Fig. 1.

The preliminary injection of large volumes of air (300-400 ml) into the celiac cavity caused the leukocyte reaction to the parenteral milk injection to be greater, not smaller, in the experiment than in the control (Fig. 2, Table 2).

Table 2 shows that the average numerical increase of leukocytes 2, 3 and 4 hours after the milk injection was 15% higher in the experiments than in the control.

Therefore, under identical experimental conditions, pneumoperitoneum in some cases (when small volumes of air are injected into the celiac cavity) decreases leukocytosis, but in other cases (when large volumes of air are injected) has a completely opposite effect and even somewhat intensifies leukocytosis.

SUMMARY

The effect of air injection into the abdominal cavity in aseptic leukocytosis caused by intramuscular injection of boiled skim milk was studied.

One group was injected with 100-150 ml of air, the second with 300-400 ml.

The leukocytic reaction depends on the volume of air injected: a small volume lowers and a large volume raises the leukocytic reaction in comparison with the control group.

Thus, a relatively small volume of air injected into the abdominal cavity inhibits reflex mechanisms effecting the leukocytic reaction following parenteral injection of milk.

LITERATURE CITED

[1] R. L. Volynskaya, in the book: The Mechanisms of Pathological Reactions," Leningrad, 1941, 3, p. 25.

[2] R. L. Volynskaya, in the book: The Mechanisms of Pathological Reactions," Leningrad, 1943, 6, pp. 21-26.

[3] E. L. Kan, Data Studying the Effect of the Nervous System on Blood Composition," Dissertation, Leningrad, 1953.

[4] K. G. Malysheva, in the book: The Mechanism of Pathological Reactions," Leningrad, 1950, 16-20, pp. 128-150.

" In Russian.

[5] K. G. Malysheva, On the Mechanism of the Leukocyte Reaction, Conditioned Reflex Leukocytosis, Leukocyte Reaction in Narcosis Caused by Barbiturates,* (Experimental study), Author's Abstract of Dissertation, Leningrad, 1951.

[6] K. A. Pimenova, in the book: Concerning the Role of the Nervous System in the Pathogenesis and Treatment of Tuberculosis,* Moscow, 1954, 1, pp. 309-319.

[7] K. A. Pimenova, Arkh. Patol., 1955, Vol. 17, No. 3, pp. 3-8.

[8] V. N. Chernigovsky and A. Ya. Yaroshevsky, Problems Concerning the Neural Regulation of the Blood Systems,* Moscow, 1953.

[9] M. D. Shkolnikova, Byull. Eksptl. Biol. i Med., 1952, Vol. 34, No. 6 (12), pp. 24-26.

[10] M. D. Shkolnikova, Byull. Eksptl. Biol. i Med., 1953, Vol. 35, No. 6, pp. 22-24.

[11] A. Ya. Yaroshevsky, Arkh. Patol., 1951, No. 3, pp. 16-20.

* In Russian.

THE DYNAMICS OF BLOOD ASCORBIC ACID IN VARIOUS SHOCK STATES.

L. A. Kashchevskaya

From the Biochemical Laboratory (Chief – Prof. L. A. Kashchevskaya) of the Scientific Research Institute for Experimental Surgical Apparatus and Instruments (Director – M. G. Ananiev)

(Received January 21, 1956. Presented by Academician A. D. Speransky)

In the literature are statements to the effect that the introduction into sensitized animals (white mice, guinea pigs, rabbits, dogs) of ascorbic acid will either completely protect them from anaphylactic shock, or will markedly retard its development [4,5,6 and others]. A whole series of authors assert that during the period of sensitization all body depots of ascorbic acid (in the future designated AA) are exhausted and that systematic saturation of the organism with ascorbic acid, before the introduction of the decisive dose of antigen, will preserve the animal's life even when the injected dose is several times larger than the one usually lethal. Other investigators assert that AA exerts only a "fleeting" antishock effect and that its influence is independent of the amount of AA in the organism, its balance or degree of saturation. These observers maintain that onset of shock can be prevented by the use of AA only when it is used 15-45 minutes before introducing the reacting dose of anitigen and that anticipatory saturation with the vitamin or its introduction $2\frac{1}{2}$ - 3 hours (200-300 mg dose of AA) previously had no effect upon the subsequent anaphylactic reaction. There are studies according to which AA has no desensitizing properties and has no influence upon the development of the Arthus–Sakharov phenomenon in animals, even if it does aid subsequently in the period of tissue defect repair [3]. There are even some studies [1] which seem to indicate that preliminary injection of AA accentuates the shock response to the antigen injection as contrasted with controls. All these contradictions mean that AA metabolism within the organism during sensitization and anaphylactic shock has not been adequately studied and this is the reason for the conflicting views. It must be noted that clinical observations seem to indicate that AA aids in removing allergic reactions. For example, inflammatory allergic responses seem to be counteracted by internal administration of AA; the therapeutic effect of AA with serotherapy against diphtheria is well described, as well as its use in hay fever and bronchial asthma. Along with other substances, AA is recommended in traumatic shock (I. P. Petrov), it is a component of the antishock solution of B. K. Kulagin, and so on. For all these reasons a more fundamental study of the indicated problems has both a theoretical and a practical interest.

In the present study we set ourselves the problem of examining what effect sensitization and anaphylaxis has upon AA metabolism and then observing what effect loading the organism with this vitamin has upon the manifestations of anaphylactic shock. In addition, AA dynamics were traced in cases of shock arising from peptides, trauma, transfusions with antagonistic blood, and fatal electric shock. We also followed through in observing what alterations in blood AA levels occurred during shock states when various portions of the nervous system were stimulated. The experimental animals were dogs (27 of them).

EXPERIMENTAL METHODS

After maintaining the dogs on a standard diet for three weeks, their blood AA levels were followed for a similar length of time, using the indophenol method for the reduced form of AA and the hydrogen sulfide method of S. D. Balakhov and L. A. Kashchevskaya for the dehydroform of AA. The animals were then sensitized to normal horse serum by using 0.5 cc of the serum per 1 kg weight for 3 days, while continuing observation of their AA levels. On the 19-21st day after sensitization the reacting dose of antigen (calculated as 1 cc

of normal horse serum per 1 kg of weight) was introduced. The arterial blood pressure and respiration of the animal was recorded on a kymograph. The AA levels were determined by taking blood from both the femoral artery and vein before the introduction of the reacting dose of antigen, at the height of shock, within 20-30 minutes, after 1 hour, 2, 24, 48 and 72 hours. The AA load and other special individual experiments are detailed below.

EXPERIMENTAL RESULTS

Our data seemed to show a tendency of the AA levels to drop during the period of sensitization, but similar fluctuations in blood levels of AA were seen in the control animals during the same 21 days. It follows that no characteristic changes in blood AA levels could be said to have definitively been observed.

When the reacting dose of antigen was introduced into the sensitized dogs, the AA content of the blood showed definite changes. In the first 1-3 minutes of the appearance of shock and at its height the AA level fell, subsequently rising to heights exceeding by several fold the original base level. In individual instances shock induced a prolonged drop in AA levels, there still not being a return to base level even after 72 hours. The succeeding series of observations were made on 6 sensitized dogs of which 4 received, during the 7 days preceding the introduction of the reacting dose of antigen, a daily 0.5 g dose of AA. The last amount of AA was given 24 hours before the experiment. Two dogs which did not recieve the excess of ascorbic acid served as controls. As a consequence, one dog of the 4 receiving AA did not manifest a shock reaction, while the other three perished in deep shock. The control animals also developed deep shock, as a result of which one died.

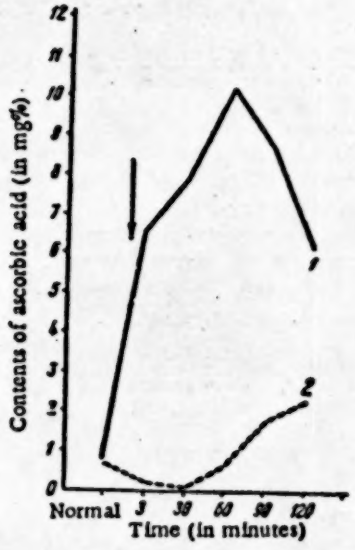

Fig. 1. Changes in blood ascorbic acid during shock. 1) venous blood; 2) arterial blood; ↓ introduction of reacting dose of antigen.

The AA levels in the blood showed the same changes as were seen in animals not receiving the saturating quantities of AA. The dog not exhibiting shock manifested, within 3 minutes, a rise in blood AA from 0.11 to 0.43 mg %, not showing a return to base level for 24 hours, while after 48 hours the AA level of the blood fell to 0.08 mg %. In these experiments we noticed that saturating the animals with AA was not reflected in the blood level, in spite of the large doses of the vitamin. For the control, nonsensitized, animals the findings were similar. To clarify this problem, we performed some special studies on dogs with exteriorized ureters (operated by method of L. A. Orbeli). We demonstrated that AA, when given to the dogs orally or subcutaneously, began to be excreted intensively in the urine within 10-15 minutes of being introduced. Because of this observation, the following series of experiments was conducted on sensitized dogs which had received AA in doses of 1-1.5 g from 15-50 minutes before receiving the reacting dose of antigen. The results are represented in Table 1. The indicated method of saturating the animals with AA did not protect them from the development of severe anaphylactic shock, leading to the death of all 7 dogs. Quite interesting is the observation that, in response to the reacting dose of antigen, dogs saturated with AA within even 1½ - 3 minutes showed a sharp rise of the reduced form of AA in the venous blood (see Figure), while in the arterial blood its quantity diminished further by the 30th minute and began to rise only after an hour, while at the same time the venous blood was being literally "flooded" with AA. In dogs that did not receive the vitamin just before the experiment, no such sharp arteriovenous differential was seen. The amount of dehydroascorbic acid in the blood during the period of the shock reaction dropped sharply, reaching minimal figures in both the arterial and venous blood, which is possibly to be explained by the upset of the oxidizing-reducing tissue activities during the shock state.

In dogs dying of anaphylactic shock, the blood of the right heart was taken immediately for studies. All

TABLE 1

Content of Ascorbic Acid (AA) in Arterial and Venous Blood of Sensitized Dogs Under Influence of a Reacting Dose of Antigen (mg %)

Dog No.	Condition of saturation with ascorbic acid	Before introducing AA	Before introducing antigen		After introduction of the reacting dose of antigen														
					1-5 minutes		10 minute		30 minutes		1 hour		1½ hour		2 hours		3 hours		
		venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	arterial blood	venous blood	
12	Not done . . .	—	0.18	0.36	0.15	0.15	0 13	0.21	0.13	0.14	0.24	0.30	0.18	0.33	0.43	0.43	0.12	0.34	Deep shock, death in 10 hours
13	Same	—	—	0.29	0.22	0.46	—	—	0.22	0.41	Died								Deep shock, death in 1 hour
14	50 min. before introducing antigen, 1.0 g AA (0.5 subcutaneously and 0.5 orally)	0.11	0.17	0.46	0.18	6.20	—	—	0 12	7.98	0.53	11.03	1.38	9.54	1 71	7.28	—	—	Deep shock, death after 6 hours
15	Same . . .	0.19	0.13	0.37	0.22	0.85	—	—	0.12	1.32	0.20	2.45	—	—	0.36	2.66	0.13	2.85	Deep shock
16	" "	0.06	0.14	0.36	0.17	0.55	—	—	0.15	7.54	Died								Deep shock, death after 50 minutes
17	" "	0 55	0.12	0 18	0.09	0.59	—	—	0.16	1.89	0.09	1.27	—	—	0.13	1.48	0.17	0.92	Deep shock, death after 4 hours
18	45 min. before introducing antigen, 0.5 g AA subcutaneously, 0.5 g orally; 15 min. previously 0.5 g intravenously	0.5	3.69	5.48	3.26	11.40	—	12.55	Died										Deep shock with death in 30 minutes

cases showed a high AA content. To establish the specific nature of this phenomenon occurring at death from anaphylactic shock, we made special studies of the blood of dogs killed with electric current. The results of these studies are shown in Table 2.

TABLE 2

Ascorbic Acid Blood Levels in Dogs Dying of Anaphylactic Shock and of Shock Produced by Electric Current

Dog No.	Ascorbic acid, in mg%		Experimental conditions
	before death	after death	
1	0.11	0.65	Death induced by electric current shock
2	0.07	0.4	Same
3	0.08	0.58	" "
4	0.39	0.75	" "
5	0.23	0.53	" "
6	0.61	1.50	Death induced by anaphylactic shock
7	0.19	1.63	Same
8	0.28	1.26	" "

As can be seen, there were sharp alterations in AA blood levels produced by either procedure. Anaphylactic shock death raised blood AA levels 2-3 times higher than did electrocution-induced shock.

In an attempt to approach an explanation of the mechanism of the observed events, we studied AA dynamics in the blood when a whole series of stimuli of various areas of the nervous system was used under shock conditions (7 dogs).*

Experiment No. 1. Dog sensitized to normal horse serum. First blood test taken before injection of the reacting dose of antigen (see Table 3). After introducing 5 cc of normal horse serum at the height of the shock reaction, both vagi were severed in the cervical region and Test No. 2 was drawn. After this, the sciatic nerve was stimulated electrically (pain reaction) and Test No. 3 was done. After an interval of time, the brain was stimulated by applying the current to the peripheral region of the carotid artery, and then Test No. 4 was taken. Still later, the medulla was stimulated electrically and blood for Test No. 6 was taken from the right heart.

TABLE 3

Dynamics of Ascorbic Acid in Blood of Experimental Dogs

Experimental conditions	Ascorbic acid mg%					
	test	test	test	test	test	test
Anaphylactic shock against the background of stimulation of nervous system with electric current	0.6	0.49	0.52	0.55	0.39	0.75
	0.21	0.26	0.23	0.53	—	—
Peptone shock	0.25 0.33	0.21 0.30	0.22 0.35	— —	— —	— —
Hemo-transfusion shock . .	0.31 0.33	0.14 0.30	0.20 0.30	— —	— —	— —
Traumatic shock	0.57	0.36	1.09	—	—	—

* The animals used were those from experiments by V. S. Kiselev and T. M. Migina (Experimental division MONIKI).

In response to the introduction of the reacting dose of antigen, the AA level of the blood fell (see Table 3) and shock followed, none of the succeeding stimuli having much effect on the AA level, the series of events being much as in the first series. Only stimulation of the medulla was accompanied by a noticeable fall in blood AA (Test No. 5).

Experiment No. 2. Dog sensitized to normal horse serum. Test No. 1 was taken at 11:40 A.M., being followed at short intervals by electrical stimulation of the sciatic nerve, sympathetic chain, cervical portion of vagus and the recurrent nerve. At 12 noon 7 cc of normal horse serum was injected. Anaphylactic shock ensued. At the height of shock, the vagi and sympathetics were severed and Test No. 2 was taken, the arterial blood pressure being 40 mm, after which the medulla and sciatic nerve were stimulated and Test No. 3 was drawn. Test No. 4 was obtained after the death of the dog by electrocution.

In this experiment it might be stated that stimulation of the nervous system prior to giving the reacting dose of antigen seemed to somewhat level out those metabolic alterations in AA which we observed in anaphylactic shock not accompanied by these additional stimuli. However, none of the manipulations performed were reflected in blood AA produced after death from electric current (Table 3).

The dynamics of blood AA during peptone shock is reflected in Experiments Nos. 3 and 4.

Experiment No. 3. The first blood test was done at 12:55, before introducing at 1:04 into the femoral vein 12.5 cc of a 25% solution of peptone. Profound shock ensued, the blood pressure dropping from 120 mm mercury column to 34 mm. At the height of shock, Test No. 2 was taken, while Test No. 3 was drawn after the animal emerged from shock.

Experiment No. 4. The first blood test was done before the experiment, blood pressure being 124 mm mercury column. After this, 17.5 cc of 25% solution was injected. In the ensuing shock, the arterial pressure fell to 54 mm. At the height of shock, Test No. 2 was taken. Then the medulla was stimulated and Test No. 3 taken. The results are also given in Table 3.

The dynamics of shock due to heterohemotransfusion is given in Experiments Nos. 5 and 6.

Experiment No. 5. The arterial pressure of the dog was 150 mm basally. At 11:15 the first blood test was done. At 11:28 75 cc of human blood, type II, was injected. Shock supervened; arterial pressure fell to 46 mm. The second test was done at the height of shock. By 11:45, when the dog had emerged from shock, the third test was done.

Experiment No. 6. The dog's basal arterial pressure was 166 mm. At 1:00 the first test was done and at 1:09 37 cc of human blood, type II, was injected. Shock developed; arterial pressure fell to 62 mm, at which time the second test was done. At 1:15 the medulla was stimulated. By 1:25 the dog had emerged from shock, the arterial pressure being 140 mm, at which time the third sample was drawn.

The results of these experiments are rather similar to those of Nos. 3 and 4 (see Table 3).

Experiment No. 7. The dynamics of ascorbic acid during traumatic shock were observed in Experiment No. 7. The dog's basal arterial pressure was 150 mm. At 12:20 the first blood test was done. At 12:30 the region of the stomach was traumatized (assayer tongs holding a weight of 1.5 kg were fastened to the stomach). At 2:30 the blood pressure was down to 40 mm, the dog going into severe shock, at which time the second test was done. As respiration ceased, the medulla was electrically stimulated. Respirations resumed. At 2:50 the arterial pressure—44 mm, rhythmic breathing, quite deep, at which time the third sample was drawn. The dog emerged from shock. The results are shown on Table 3.

It can be seen that traumatic shock produces marked changes in the blood ascorbic acid level.

DISCUSSION OF RESULTS

The mechanism of the observed changes of blood ascorbic acid in shock states seems to present complexities. The "flooding" of venous blood by ascorbic acid in shock states of animals, previously saturated with vitamin C, and the lower content of the vitamin in the arterial blood suggest that ascorbic acid, apparently, enters the blood from all tissues and not just from special depots (adrenals, liver, etc.). Study of the arteriovenous differences in the femoral vessels rather proves that muscular tissues are large participants. We believe

that shock states liberate ascorbic acid from its combination with protein – ascorbigen. This process we described in a previous study when we traced the effects of excessive cerebral cortical stimulation by exteroceptive stimuli upon ascorbic acid metabolism within the tissues. In the first phase of shock – phase of excitement – there appears to be an intensive utilization by the tissues of the ascorbic acid which is simultaneously being liberated into the venous blood. In the depressed phase the latter process predominates. We also make the assumption that ascorbic acid enters the lungs, where, under conditions of increased aeration, there occurs a certain amount of decomposition of this vitamin. This again leads to the arterial blood having less ascorbic acid than the venous. We can ascribe the higher content of ascorbic acid in cases of anaphylactic shock death as compared to cases dying from electric shock also, in part, to the fact that there is greater venous stasis resulting from the much longer vascular dilatation seen in anaphylactic shock.

SUMMARY

It was demonstrated that the pronounced shifts in the level of reduced blood ascorbic acid are observed during anaphylactic or traumatic shock. In the first phase the ascorbic acid level decreases rising during the depression phase especially in the venous blood. Studies of the arterio-venous differences in the femoral vessels demonstrated that the muscular tissue is considerable part taking in this process releasing large quantities of ascorbic acid. Irritation of the peripheral nervous system at a time when shock has already developed does not change markedly the ascorbic acid in blood. At the period of sensitization no special changes were noticed in the metabolism of ascorbic acid. No method of saturating sensitized animals with ascorbic acid did prevent the development of anaphylactic shock.

LITERATURE CITED

[1] V. P. Bezuglov and E. N. Domontovich, in the book: Allergy and Desensitization,* Kharkov, 1949. pp. 41-53.

[2] L. A. Kashchevskaya, Byull. Eksptl. Biol. i Med., 1954, Vol. 33, No. 3, pp. 37-41.

[3] N. I. Speransky and V. I. Ostrovskaya, Byull. Eksptl. Biol. i. Med. 1952, Vol. 33, No. 5, p. 38.

[4] M. K. Ebert, Zhur. Mikrobiol. Epidemiol. i Immunobiol., 1949, No. 6, p. 72.

[5] A. Hochwald, Fiziol. Zhur. SSSR, 1936, Vol. 21, No. 5-6, pp. 942-944.

[6] A. Hochwald, Klin. Wschr., 1936, No. 25, pp. 894-898.

* In Russian.

PHARMACOLOGY

THE INFLUENCE OF CHLORPROMAZINE AND PROMETHAZINE ON VISCERO-VISCERAL REFLEXES

B. A. Medvedev

Department of Pharmacology (Chief – Professor M. D. Mashkovsky), All-Union S. Ordzhonikidze Institute for Chemico-Pharmaceutical Research, Moscow

(Received July 24, 1956. Submitted by Active Member of the Academy of Medical Sciences of the USSR Prof. V. V. Zakusov)

The study of substances which exert an influence on viscero-visceral reflexes is of great theoretical and practical importance.

In the literature there are communications which deal with the influence of certain pharmacological agents on these reflexes. N. V. Kaverina [1,2,3] found that local anesthetic (Novocaine, Dicaine, etc.) and narcotic substances (Medinal, urethane) and also glucose reduce the cardiovascular and respiratory responses to stimulation of the internal organs. R. P. Kruglikova-Lvova [4] observed that morphine and, to a lesser degree, Promedol, intensify the pressor reflex from the urinary bladder and the large intestine.

Our object was to study the influence of chlorpromazine (Largactil, Megaphen) and promethazine (Phenergan) on the reflexes elicited by stimulation of certain internal organs.

Courvoisier et al., [6] report briefly on the suppressive effect of Largactil on the reflexes induced by occluding the common carotid artery and by stimulating the ischiatic nerve. Analogous observations have been made by M. D. Mashkovsky, S. S. Liverman and A. I. Polezhaeva [5]. Kalkhoff [7] noted suppression following administration of Megaphen of the pressor reflexes induced by occluding the carotid artery. There has been no study made to date on the influence of chlorpromazine on other interoceptor reflexes. We have been unable to discover any published findings on the effect of promethazine on viscero-visceral reflexes.

EXPERIMENTAL

The experiments were conducted on cats under deep urethane or urethane-Nembutal narcosis. Urethane was administered intraperitoneally in the majority of experiments, but in some it was injected intravenously or intramuscularly.

The doses of urethane varied from 1.3-1.5 g/kg depending on the individual sensitivity of the animals. In the experiments in which anesthesia was produced by urethane plusNembutal, urethane was administered at a dose of 1.0-1.1 g/kg together with 10 mg/kg Nembutal.

Stimulation of the interoceptors of the urinary bladder and small and large intestinal ansae was effected by inflating these organs with air at a pressure of 50-80 mm Hg. The ischiatic nerve was stimulated by a current from an induction coil, the distance between the primary and secondary coils being 11-13 cm. Stimulation of the baroceptors of the carotid sinus was induced by occluding the common carotid artery by means of a Dieffenbach clamp.

In all the experiments, blood pressure was recorded by means of a mercury manometer connected to the common carotid artery and respiration by a Marey capsule connected to the trachea.

Stimulation was applied both before administration of chlorpromazine and promethazine and the following

intravenous injection of these substances, usually after a time lapse of 1-3, 5, 15, 30, 60 and 120 minutes, but in some experiments even after 3, 4, 5 or 6 hours. 60 animals were used in the investigation.

RESULTS

In the experiments in which the urinary bladder was stimulated, it was found that at doses of chlorpromazine as low as 0,05-0,1 mg/kg the reflex rise in blood pressure was reduced by 20-30% and in some animals by 50% as compared with the level of the original reflex. The duration of action of these doses of chlorpromazine was 1½ to 2 hours (Fig. 1).

The action of promethazine proved to be analogous in intensity and duration at doses of 1-5 mg/kg and at higher doses. Lower doses of promethazine often either exerted no effect or even produced increase in the reflex rise in blood pressure.

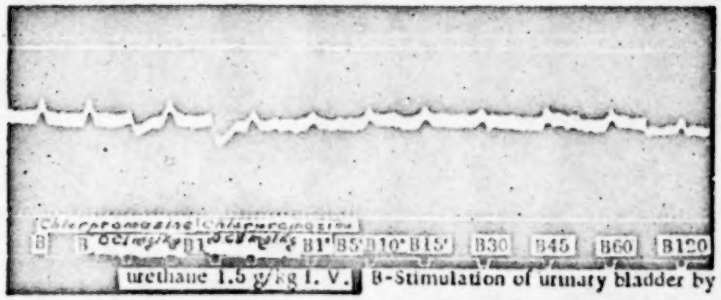

Fig. 1. Influence of chlorpromazine on vasopressor reflex with stimulation of urinary bladder. Cat 4 kg. Urethane 1,5 g/kg intravenously. Tracings from top to bottom: respiration, blood pressure, time (15 seconds), periods of stand-still of kymograph in minutes, stimulation of urinary bladder before administration and 1, 5, 10, 15, 30, 45 and 60 and 120 minutes after intravenous injection of chlorpromazine at dosage of 0,05 mg/kg.

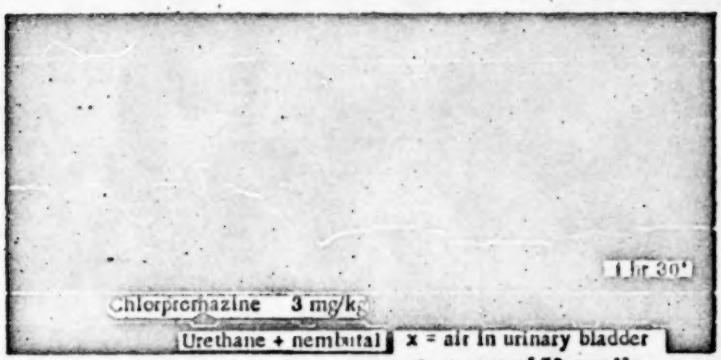

Fig. 2. Influence of chlorpromazine on vasopressor reflex with stimulation of urinary bladder. Cat 4,5 kg. Urethane 1,2 g/kg and Nembutal 10 mg/kg. Tracings as in Fig. 1. Time interval -5 seconds. Stimulation was applied 5, 20, 60 and 160 minutes after administration of chlorpromazine.

When the dosage of chlorpromazine was increased to 0,5-3 mg/kg, in the majority of experiments, there

was observed almost total suppression of reflex changes in arterial pressure, for a period of two or more hours. This was followed by slow restoration of the vasopressor reflex which did not return to the initial level for 5-6 hours (Fig. 2).

Promethazine did not exert such a powerful and prolonged effect even at a dosage of 10 mg/kg.

The respiratory changes on stimulation of the urinary bladder were in our experiments inconsistent and nonspecific. Nevertheless, it could be noted that in some experiments following administration of chlorpromazine or promethazine, the respiratory changes induced by stimulation of the interocepton of the urinary bladder became less marked.

In the experiments with stimulation of ansae of the large and small intestines it was seen that chlorpromazine at a dose of 0.01 mg/kg, while not essentially influencing the magnitude of the pressore reflex, produced a change in its character; the rise and fall in blood pressure showed greater slope. Promethazine exhibited an action roughly similar in intensity at a dose of 1 mg/kg. Smaller doses of promethazine (0.1 mg/kg) produced a short (10-15 minutes) slight intensification of the pressor reflex.

Chlorpromazine, at doses of 0.05 - 0.1 mg/kg reduced the vasopressor reflex from the intestines by 20-40% and, at doses of 0.5-1.0 mg/kg, by more than 60%, for a long period (up to 2 hours); this was followed by a slow increase but complete restoration of the initial amplitude of the reflex was not observed in any experiment (Fig. 3).

Total suppression of the pressor reflex was seen lasting for 3-5 hours and longer with doses of 3-5 mg/kg chlorpromazine.

The respiratory changes on stimulation of the intestines were in our experiments inconsistent and it is therefore difficult to assess the effect of chlorpromazine and promethazine in this respect.

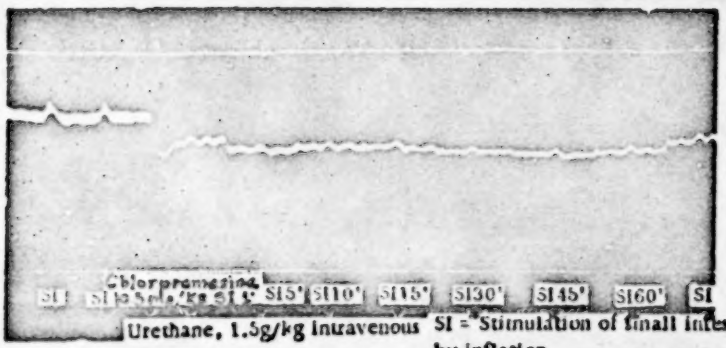

Fig. 3. Influence of chlorpromazine at dose of 0.05 mg/kg on vasopressor reflex with stimulation of small intestine. Cat 3.35 kg. Urethane 1.5 g/kg. Tracings as in Fig. 1.

Stimulation of the ischiatic nerve by an induction current in the control experiments produced a rapid rise in arterial pressure, increased frequency and a sharp increase in the depth of respiration.

Following administration of chlorpromazine at doses of 0.05-0.1 mg/kg a reduction in the pressor reflex response of approximately 50% was observed for 1-2 hours.

Promethazine exerted an action analogous in strength only at doses of 5-10 mg/kg; low doses of 0.1-0.5 mg/kg causing a distinct increase in the reflex response for 1-1½ hours following administration.

With increased dosage of chlorpromazine the vasopressor reflex decreased and at doses of 3-5 mg/kg was completely absent for several hours.

In experiments with stimulation of the ischiatic nerve in which the respiratory response was always dis-

tinctly manifest, chlorpromazine and promethazine exerted a very marked influence on reflex changes in respiration.

Chlorpromazine at doses of 3-5 mg/kg shortened the duration of increased respiratory frequency and the amplitude of respiratory movements. Doses of 0.01-1.0 mg/kg exhibited a less clearly marked action.

The doses of promethazine required to produce an effect of similar intensity were about double those of chlorpromazine.

When the baroceptors of the carotid zone were stimulated by occlusion of the common carotid artery a decrease in the pressor reflex was also seen following administration of chlorpromazine. An initial effect was observed at doses of chlorpromazine as low as 0.01 mg/kg; increase in dosage to 0.05 mg/kg led to a 50-60% diminution in the vasopressor reflex for one hour.

An effect analogous in strength was seen with promethazine at a dose of 5 mg/kg. This preparation at doses below 0.1 mg/kg did not exert an appreciable influence on the magnitude of the reflex; at a dose of 0.1 mg/kg a distinct increase was produced in the vasopressor reflex (Fig. 4).

Chlorpromazine at a dose of 3 mg/kg suppressed the reflex from the carotid sinus for a long time (5-6 hours).

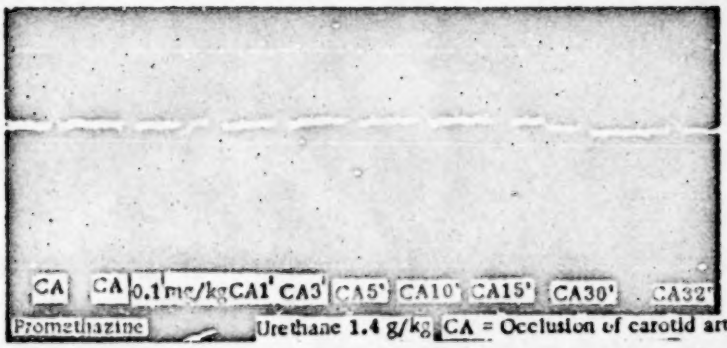

Fig. 4. Effect of promethazine at dose of 0.1 mg/kg on vasopressor reflex induced by occluding common carotid artery. Cat 4.1 kg. Urethane 1.4 g/kg. Tracings as in Fig. 1. Stimulation applied 1, 3, 5, 10, 15 and 30 minutes after administration of promethazine.

In our experiments changes in respiration with stimulation of the baroceptors of the carotid sinus were insignificant and no conspicuous effect on these changes was exerted by chlorpromazine or promethazine.

DISCUSSION OF RESULTS

Our findings demonstrate that the representatives of the phenothiazine series tested by us are able to decrease or suppress the reflex changes in blood pressure produced by stimulation in experimental conditions of internal organs.

This effect was particularly marked in the case of chlorpromazine which, at doses of 0.01 mg/kg and less, changed the character of the pressor reflexes and at higher doses either sharply reduced or completely removed them.

Promethazine also reduced the reflex blood pressure responses but its activity was ten to twenty times lower than that of chlorpromazine and the duration of its action was considerably less. Furthermore, with use of promethazine we observed a feature not seen with chlorpromazine, i.e., small doses of promethazine (0.1 mg/kg) produced intensification of the vasopressor reflex with significant regularity.

The compounds under investigation also exerted an influence on reflex changes in respiration, but this

effect could not be demonstrated so easily and was less uniform in character.

In comparing our results with published findings on substances which have been tested for influence on vasopressor reflexes, it will be seen that chlorpromazine shows significantly higher activity than do other substances. Thus, according to N. V. Kaverina Novocaine at doses of 2-3 mg/kg reduces vasopressor reflexes by 20-30% whereas an analogous effect is seen with chlorpromazine at doses of 0.05-0.1 mg/kg, i.e., doses at least 30-40 times less. Other compounds (glucose, urethane, Sovcaine, Dicaine, etc.), according to the same author, are either less active or produce a powerful toxic effect. Thus, at present chlorpromazine is apparently one of the most active substances known possessing the property of suppressing interoceptor reflexes.

The particular aspect of activity of chlorpromazine investigated by us is apparently to be explained by various factors.

First of all, it is necessary to take account of the blocking (neuroplegic) action of this compound. Its ability to exert an influence on the different links in the nervous system cannot but affect the conduction of excitation in the central link of the interoceptor reflex arc. However, of great importance in the action of chlorpromazine is the influence which it exerts on the peripheral links of the reflex arc, in particular, the blocking effect of the preparation on the adrenoreactive systems which receive the vasoconstrictor impulses. This is all the more likely since the doses of chlorpromazine at which the adrenolytic effect starts to manifest itself coincide with the doses which produce initial changes in the reflexes (0.01-0.05 mg/kg).

The lower activity of promethazine must, it seems, be explained by its less powerful central blocking (neuroplegic) and also its lower adrenolytic action as compared with chlorpromazine. Since the antihistamine activity of promethazine considerably exceeds that of chlorpormazine it must be considered that this type of pharmacological action is not directly connected with influence on interoceptor reflexes.

SUMMARY

The effect of chlorpromazine and promethazine on viscero-visceral reflexes caused by stimulation of the interoreceptors of the urinary bladder, intestine, sinus caroticus, as well as by stimulating the ischiadic nerve was studied. Both substances can inhibit these reflexes. Chlorpromazine is 10 to 20 times more active than promethazine. Small doses of promethazine can enhance vasopressor reflexes.

LITERATURE CITED

[1] N. V. Kaverina, Farmakol. i Toksikol., 1951, XIV, No. 2, pp. 20-24.

[2] N. V. Kaverina, Farmakol. i Toksikol., No. 2, pp. 17-22.

[3] N. V. Kaverina, Farmakol. i Toksikol., XV, No. 3, pp. 18-22.

[4] R. P. Kruglikova-Lvova, Farmakol. i. Toksikol., 1953, XVI, No. 1, pp. 8-14.

[5] M. D. Mashkovski, S. S. Liberman and A. I. Polezhaeva, Farmakol. i Toksikol. XVIII, No. 1, pp. 14-22.

[6] S. Courvoisier, and others, Arch. intern. pharmacodynamie, 1953, Vol. 92, No. 3-4, pp. 305-361.

[7] W. Kalkhofi, Arch. f. exper. Pathol., Pharm., 1955, Bd. 225, S. 92-95.

THE EFFECT OF EPILEPTOGENIC EXCITANTS—CAMPHORATED OIL AND PYRAMIDON (AMIDOPYRINE) — ON CHEMORECEPTORS OF THE SMALL INTESTINE

O. S. Merkulova

From the Laboratory of Pathological Physiology (Chief: Prof. V. S. Galkin) of the I. P. Pavlov Institute of Physiology (Director – Academician K. M. Bikov), Academy of Sciences USSR, Leningrad

(Received February 29, 1956. Presented by Active Member of the Academy of Medical Sciences USSR, Prof. V. N. Chernigovsky)

I. P. Pavlov considered that an outburst of excitation in an isolated pathological point of the cerebral cortex was the basis of a human epileptic seizure. This point – differing from one patient to another – should not necessarily be considered as a gross morphological lesion. It may also arise as a result of functional disturbance, or even merely be the sequel of the breakdown of a dynamic complex [5]. The outburst of excitation at the pathological point originates as a result of an afferent impulse from the periphery, i.e., by the reflex path.

Reflex provocation of a seizure can be illustrated clinically by extensive case material. However, to date, the reflex nature of the mechanism has been demonstrated experimentally only to a small extent [3,6,7,8,9,-11,12].

The reflex nature of a seizure stands out clearly in so-called "bell epilepsy" [3]. Material on the reflex nature of an epileptic seizure is given in the works of V. S. Galkin and co-workers (see summary in dissertation of L. P. Kokorev, 1952).

For the purpose of inducing experimental epilepsy, many of the chemical epileptogenic agents have been administered parenterally.

In order to reveal the mechanisms in the development of epilepsy induced by such substances it seemed to us necessary to answer the following question: "what influence do these substances exert on the interoceptors with which they inevitably come into contact on entering the organism?"

To begin with, we studied the influence of two epileptogenic excitants – camphorated oil and Pyramidon – on the chemoreceptors of the small intestine. It seemed to us essential to conduct experiments along these lines in order to make a fuller and complete analysis of the effect on the organism of these substances, which as is well known are widely used in therapeutic medicine.

EXPERIMENTAL

20 experiments were performed on cats, anesthetized by means of intravenous injection of urethane, with perfusion of a segment of the small intestine according to the method of V. N. Chernigovsky. We studied the reflex influence on blood pressure in the carotid artery and on respiration following administration of camphorated oil and Pyramidon into the vessels of the perfused segment of the small intestine. We made use of a 20% solution of camphor in peach oil and a 4% aqueous solution of Pyramidon. It proved necessary to verify in subsequent experiments the influence of these substances on the excitability of the chemoreceptors of the small intestine. For this purpose nicotine at doses of 50-200 γ was injected into the vessels of the intestines before and after the chemoreceptors had been subjected to the influence of camphorated oil and Pyramidon.

EXPERIMENTAL RESULTS

On administration of camphorated oil into the vessels of the small intestine, pressor reactions were seen (in blood pressure) and respiration was stimulated (Fig. 1). These pressor reactions were of a protracted "tonic" character. The threshold doses required to bring about these reflexes amounted to 2-4 mg camphor. With increased dosage of the excitant the pressor effect at first grew in amplitude but later only a protraction of the effect was observed. In one experiment out of six, reflex responses involving skeletal muscles – so-called "actuating" influences – were obtained.

Denervation of the perfused intestinal segment and also preliminary injection of novocaine into the intestinal vessels removed reflex influences. After the novocaine had been washed off with nutrient solution the reactions were restored.

In order to ensure that the results obtained were not the consequence of an oil embolism, we conducted control experiments in which we injected peach oil – the solvent of camphor in the preparation of camphorated oil – into the vessels of the perfused intestinal segment.

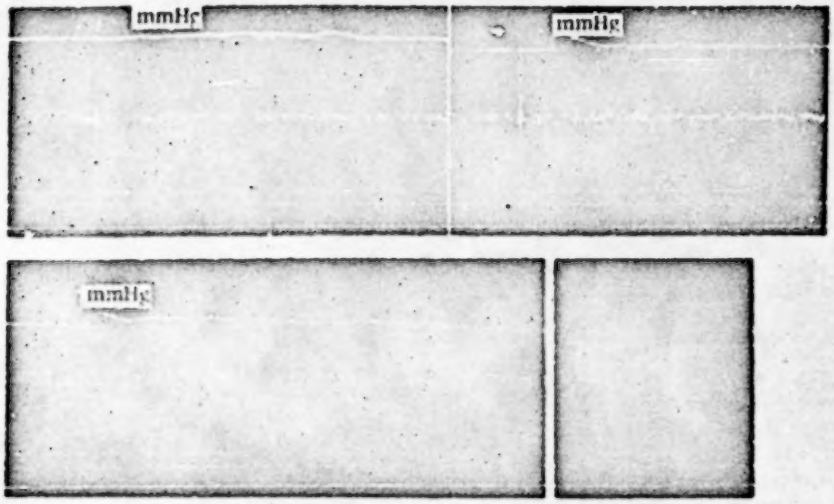

Fig. 1. Stimulation of chemoreceptors of small intestine in cats by camphorated oil. Tracings – top to bottom: blood pressure in carotid artery, line of original level of blood pressure, respiration, base line for mercury manometer, indication of stimulation, time interval (5 sec). Administration of camphorated oil: 1) 12 hours 3 minutes, 0.01 ml; 2) 12 hours 15 minutes, 0.05 ml; 3) 12 hours 32 minutes, 0.2 ml; 4) 12 hours 55 minutes, 0.2 ml peach oil.

Introduction of appropriate quantities of peach oil was not accompanied by reflex effects on blood pressure and respiration (Fig. 1,4).

Another interesting feature of these experiments was that following administration of peach oil the blood pressure reflex reactions in response to administration of camphor were extremely protracted. Thus, in the experiment of December 4, 1955, 40 mg camphor introduced 17 minutes after administration of 0.2 ml peach oil produced a rise in blood pressure for 12 minutes.

In six experiments, we made a study of the influence of camphorated oil on the excitability of the chemoreceptors of the small intestine, the excitability being determined according to reflex responses to nicotine. Following administration of camphorated oil at doses of 0.1-0.5 ml (20-100 mg camphor), prolonged inhibition of reflex responses to nicotine was observed (Fig. 2), and in some experiments (with use of large doses) there was

Fig. 2. Influence of camphorated oil and peach oil on excitability of chemoreceptors in cat. Tracings - as in Fig. 1. 1) Administration 50 γ nicotine before administration of camphorated oil; 2) administration of 0.2 ml camphorated oil; 3,4,5,6,7) repeat administration of nicotine following administration of camphorated oil - 50 γ per administration.

even irreversible inhibition - the reflexes were not restored at all during 1-2 hours. Even when they were restored, the responses to nicotine were sometimes very protracted. Small doses of camphorated oil (0.03-0.05 ml) did not exert an inhibitory effect on reflex responses to nicotine; however occasionally within 20-30 minutes the responses became very protracted in character. It is characteristic that camphor, while inhibiting reflex responses to nicotine, did not influence the responses to repeated administration of camphor itself.

No inhibition of reflex responses to nicotine was observed following administration of peach oil.

For comparison purposes, camphorated oil was introduced into the femoral vein and always a depressor response was seen in blood pressure and inhibition of respiration. This fact is not new; it was established in the work of V.G. Baranoff and E. N. Speranskaja [10] and confirmed in the work of A. G. Bukhtiyarov [1].

Introduction of even very large doses of Pyramidon (up to 150 mg) into the vessels of the perfused segment of the small intestine did not produce distinct changes in blood pressure and respiration. However, as verified by the reactions to nicotine, the excitability of the intestinal chemoreceptors was subject to considerable change. There was either complete disappearance or marked inhibition of the reflex responses to nicotine after treatment of the intestinal receptors with Pyramidon (Fig. 3). 5-10 minutes after washing off the Pyramidon, reflex responses to nicotine were completely restored. Analogous results were obtained not only with administration of a single specific dose of Pyramidon (40-150 mg), but also when the liquid used to perfuse the small intestinal segment was changed from the normal Tyrode solution to this same solution plus a given amount of Pyramidon so that, for example, a 100 mg % Pyramidon solution was obtained.

In two experiments out of six, following introduction of Pyramidon (40-60 mg), a very insignificant decrease in the magnitude of the reflex responses to nicotine was observed. However 15-30 minutes after this there occurred an extreme prolongation of the pressor responses to nicotine - up to 6 minutes. Within 40-50 minutes the responses were again shortened.

The experiments conducted demonstrate that the interoceptors have a particular affinity for both substances investigated by us - camphorated oil and pyramidon - and that these substances exert on the interoceptors a completely specific effect.

Camphorated oil powerfully stimulates the chemoreceptors of the small intestine, the active factor being camphor and not the peach oil used as solvent. Furthermore, camphorated oil exerts a powerful inhibitory effect

on the excitability of the chemoreceptors of the small intestine. The active principle here also is camphor and not the peach oil.

Pyramidon at doses up to 150 mg although it does not itself produce distinct reflex changes in blood pressure and respiration, considerably diminishes the excitability of the chemoreceptors to nicotine. These findings are in agreement with the generally known sedative action of Pyramidon.

The protracted reactions in blood pressure observed in a number of the experiments are of particular interest. An analogous effect was observed by us in joint work with M. Ya. Ratner (1955) by treating the chemoreceptors of the small intestine with renin and by V. A. Lebedeva (1955) in work with Pentothal with application of the same experimental methods. Prolonged blood pressure reactions were observed by T. P. Yakimova in 1952 in experiments on cats with introduction of hot solution of alcohol (32°) and sodium chloride (20%) into the carotid artery and femoral vein. We venture to advance the hypothesis that the development of prolonged reactions of the "tonic" type from the chemoreceptors of internal organs is a type of general phenomenon the mechanism of which must be subjected to more thorough experimental analysis.

Fig. 3. Suppression of reflex responses from chemoreceptors of small intestine by Pyramidon in cats. Tracings – as in Fig. 1. 1) 13 hours 30 minutes – administration of 100 γ nicotine; 2) 13 hours 34 minutes – administration of 80 mg Pyramidon; 3) 13 hours 35 minutes – administration of 100 γ nicotine accompanied by decreased blood pressure reaction; 4) 13 hours 40 minutes – administration of 100 γ nicotine. Amplitude of reaction completely restored.

On the basis of the experiments conducted, it is possible to postulate that in the development of camphor- or Pyramidon - induced experimental epilepsy a definite role may be played by the reflex influences arising from the action of these substances on receptors in internal organs. This is all the more probable since the threshold epileptogenic dosage in cats of these substances (40 mg for camphor and 240 mg for Pyramidon) greatly exceeds the threshold dosage necessary to influence the chemoreceptors of the small intestine (2-4 mg for camphor and 40-60 for Pyramidon).

Consequently, with application of epileptogenic doses of these substances, the receptors of the internal organs undergo the most powerful stimulation and are the sources of a large number of nerve impulses.

SUMMARY

The influence of camphorated oil and Pyramidon on chemoreceptors of the small intestine was studied on cats under urethane anesthesia. After the injection of camphorated oil into the vessels of the small intestine by the method of perfusion a reflex increase of the blood pressure and stimulation of respiration was noted. Moreover, comphorated oil inhibited reflex reactions of chemoreceptors to nicotine. Injection of Pyramidon into the vessels of the small intestine did not effect any change in blood pressure and respiration, whereas the reactions to nicotine were greatly inhibited. The author surmises that reflexes play an important part in the development of experimental epilepsy.

LITERATURE CITED

[1] A. G. Bukhtiyarov, Intra-Arterial and Intravenous Injection of Certain Chemical Excitants (Experimental Investigation),* Leningrad, 1949.

[2] L. P. Kokorev, cited by V. S. Galkin "Experiment in the Application of the Conditioned Reflex Method in Everyday Pathophysiological Practice", Mechanisms of Pathological Reactions,* Medgiz, 1955.

[3] L. V. Krushinski, Uspekhi Sovremennoi Biol. Vol. 1 (4), pp. 108-133.

[4] O. S. Merkulova and M. Y. Ratner, Byull. Eksptl. Biol. i Med., 1955, Vol. 39, No. 5, pp. 45-50.

[5] I. P. Pavlov, in the book: Pavlovian Media,* Leningrad, 1949, Vol. 3, pp. 402-405.

[6] N. N. Studentsov, cited by Ya. A. Vasilev, 1924, Vol. 6, Nos. 4-6, p. 81.

[7] L. A. Sapov, Byull. Eksptl. Biol. i Med., 1953, Vol. 35, No. 5, pp. 18-23.

[8] G. Uzunov, S. Bozhinov and I. Georgiev, Zhur. Sovremennoi Med., 1952, No. 5, pp. 28-34.

[9] G. Amantea, Zentralbl. f. Physiol. Ztvl., 1912, N. 26, S. 229.

[10] W. Baranoff, E. Speranskaya, Stepanowa E., Ztschr. f. ges. experim. Med., 1931, Bd. 78, S. 484-491.

[11] Brown-Sequard, Ch. D'une affection convulsive qui survient chez les animaux qui ont une moytie laterale de la moelle epinere coupee. Compt. rend. soc. de biol., 1851, N. 2.

[12] L. V. Laborde, Compt. rend. soc. de biol., 1891, N. 15, p. 287.

* In Russian.

MICROBIOLOGY AND IMMUNITY

ACQUIRED TOLERANCE TO FOREIGN SERUM IN RATS

I. N. Kryukova

From the Division of Immunology and Malignant Tumors (Chief - Active Member of the Academy of Medical Sciences USSR L. A. Zilber), N. A. Gamaleya Institute Immunology, Epidemiology and Microbiology, Academy of Medical Sciences USSR, Moscow

(Received November 1, 1956, Presented by Active Member of the Academy of Medical Sciences USSR, L. A. Zilber)

It has been established by the work of numerous authors that the introduction of a foreign antigen into an animal in the embryonic stage of development depresses the ability of the animal to produce antibodies to this antigen in postnatal life. Owen [7] first observed such acquired tolerance to blood of a foreign group in binovular twin calves, and Dunsford and Bowley [3] in binovular human twins. In both cases exchange of blood was established in the embryonic stage through the communication of the placental blood vessels. Billingham, Brent and Medawar [2] and Gashek [1] were able to establish tolerance experimentally in birds and mammls to normal tissues and blood of animals of other lines and furthermore to establish skin transplantation and blood transfusion between such animals. The antigens were suspensions of organs from animals from the donor line and they were injected into the recipient during the embryonic stage. The attempts of Gashek and Simonsen [8] to build up in birds and mammals tolerance to normal tissue from another species have not yet been successful.

Successful transplantation of a tumor from animals of one species into animals of another species by the aid of the technic of preliminary injection of a suspension of normal tissues from the donors into the embryos of the recipients was achieved by Koprovsky [6] and Wallace [9].

The results obtained oblige one to suppose that the phenomenon described possesses broad biological characteristics and extends to many other antigens.

Hanau and Oyama [5] and Dixon and Maurer [4] were able to lower the ability of rabbits to produce antibodies to bovine serum albumin by injecting the newborn animals. Such lowered immunological activity persisted even after all the antigen had disappeared from the organism.

In our own experiments we attempted to produce tolerance in rats to horse serum by injecting it into the embryos on the 17th – 18th day of development (period of gestation in the rat: 21 – 22 days).

METHODS AND RESULTS

We anesthetized pregnant rats with ether and cut through the skin of the abdomen, the muscle layer and the peritoneum along the midline. Through the wall of the uterus and the embryonic membrane we injected each embryo either intramuscularly or subcutaneously with 0.03 – 0.04 ml of horse serum. We used a 1 ml syringe and a No. 25 needle. In all, 10 rats were operated on. Of the progeny it was possible to save 11 young from three rats (Nos. 9, 19 and 7).

On attaining a weight of 200 g (2 – 2½ month of age) all the experimental animals and 9 control (unoperated) rats of the same weight and degree of maturity were immunized intravenously with normal horse serum in increasing doses: 0.1, 0.2, 0.3, 0.4 and 0.5 ml. We made the injections every other day.

Precipitin Reaction with Sera of Experimental and Control Rats

	Animals	No. young	Dilution of Antigen				
			1:10	1:50	1:100	1:150	1:300
Experimental	Young of Rat No. 19	59	+	±	–	–	–
		64	+	±	–	–	–
	Young of Rat No. 7	60	+	–	–	–	–
		61	+	–	–	–	–
		62	–	–	–	–	–
		63	++	–	–	–	–
Controls	Not previously injected with antigen	82	+++	+++	++	+	++
		83	+++	++	+++	+	++
		84	–	++	++	+	++
		85	++	+	+	++	+
		47	++	++	++	+	±
		48	++	+	+	+	+
		49	+++	++	+	--	--

Note. All rat sera diluted 1:2

About 7 days after the last injection blood was taken from all the rats.

Antibody to horse serum present in the sera was checked by the precipitin reaction (see Table).

In the offspring of one of the operated rats (No. 9) the ability to produce precipitins against horse serum, compared to the controls, was not reduced, which is possibly connected with technical errors in the injection of serum into the embryos. The progeny of the other two rats (Nos. 7 and 19) showed definite lowering of the precipitin titer as compared with the controls. To one of the offspring (No. 62) there may be ascribed complete tolerance to the injected antigen; precipitation is absent with a 1:10 dilution of horse serum just as with further dilutions.

Thus the results obtained confirm the principle established by other authors. A foreign antigen, injected into an organism during embryonic life, when the reticuloendothelial system is not capable of manufacturing antibodies, ceases to be an antigen for this organism. The mechanism of this phenomenon is so far not clear, and the solution of the present question demands systematic investigations along various lines.

SUMMARY

Introduction of normal horse serum into rat embryos resulted in marked depression of antibody formation to this antigen in the adult animals.

LITERATURE CITED

[1] M. Gashek, Zhur. Obshchei Biol. 1955, 16, pp. 405-412.

[2] R. E. Billingham, L. Brent, and P. B. Medawar, Nature, 1953, Vol. 172, pp. 602-606.

[3] J. Dunsford, C. C. Bowley and others, Brit. Med. Journ., 1953, No. 4827, p. 81.

[4] F. J. Dixon and P. H. Maurer, J. Exp. Med., 1955, Vol. 10, pp. 245-257.

[5] R. Hanau and J. Oyama, J. Immunol., 1954, Vol. 73, pp. 49-53.

[6] H. Koprowsky, Nature, 1955, Vol. 175, pp. 1087-1088.

[7] R. D. Owen, Genetics, 1946, N. 2, p. 227.

[8] M. Simonsen, Nature, 1955, Vol. 175, N. 4461, pp. 763-764.

[9] A. C. Wallace, Cancer Research, 1956, Vol. 16, No. 4, pp. 348-351.

THE REFLEX PRODUCTION OF ANTIBODIES CAUSED BY THE INJECTION OF ANTIGENS INTO THE ISOLATED SPLEEN

A. N. Gordienko, V. I. Kiseleva, B. A. Saakov, R. B. Tsynkalovsky, Ya. I. Azhipa, A. V. Letyen, A. I. Egorov, I. M. Bondarev and L. I. Zhigalina

From the Department of Pathophysiology (Head – Prof. A. N. Gordienko), Rostov Medical Institute

(Received November 3, 1956. Presented by Academician A. D. Speransky)

In the work which G. A. Strashun conducted in our laboratory, it was shown that the injection of an antigen into a spleen isolated from circulation and then removed, with the antigen, five minutes later causes the production of antibodies [2]. This data is confirmed by the studies of V. A. Tychinin and E. A. Tatarinov [3,4]. Recently, K. A. Pozdeev [1] concluded that isolating a spleen does not prevent an antigen injected into it from being resorbed, and that antibody production only occurs when the antigen is found in the blood.

In order to prove that antibodies are produced when an isolated spleen is injected with an antigen, which is not resorbed from the place of its injection, we decided to develop a method of isolating the spleen and, using this method, to prove that the action of the antigen on the spleen receptors causes the reflex production of antibodies.

EXPERIMENTAL METHODS

In sterile conditions, operating on dogs under anesthesia, we opened the celiac cavity, brought the spleen to the surface, separated the splenic nerve and applied a ligature. A probe was inserted between the nerves and vessels of the spleen, from its hilum to its convex part. Two ligatures were applied along the probe – one tying completely all the vessels in the upper portion of the spleen, the other tying completely all the vessels in the lower part of the spleen; these two ligatures effected the complete isolation of the spleen from the circulatory system; the only remaining connection between the spleen and the body was the intact nerve.

We verified the complete isolation from circulation of the spleen by injecting radioactive phosphorus into the spleen in a dose of 200 microcuries and then determining it in the blood by counting the impulses with a Type B dosimeter. For this purpose, blood was taken before the P^{32} injection, five minutes after the injection and simultaneously with the removal of the spleen. The number of impulses was computed several times on each batch of blood. The number of impulses did not change much and remained within the limits of normal fluctuation. This indicated that the radioactive phosphorus injected into the spleen was not absorbed and that the isolation of the spleen from general circulation was complete.

As well as radioactive phosphorus, typhoid fever vaccine was injected into the spleen, isolated by the method described above. Five minutes after the antigen injection, the spleen was removed. Blood was taken seven to eight days after the immunization, and the titer of antibodies to the typhoid bacillus and to the enteric bacillus was determined from the serum obtained from this blood.

EXPERIMENTAL RESULTS

In the control series of experiments, an analogous injection of a physiological solution into the subsequently removed spleen did not change the antibody content, although the original titer was rather large (Fig. 1, b). The

determinations showed that the titer of typhoid bacillus antibodies increased, but that the titer of enteric bacillus antibodies did not change, if the increase observed in some cases of antibodies by one dilution, which is shown in Fig. 1a is discounted.

From the data obtained, we concluded that the typhoid fever bacillus, when injected into a spleen isolated from circulation, stimulates the spleen receptors and causes the reflex production of specific antibodies. There were no antibodies to the related group produced in this case.

In the second series of experiments, a dog's spleen was isolated by the method described above, but additional ligatures were also applied to the vascular plexi, and the plexus containing vessels and cellular tissue was transected between the first and second ligatures. In this case, therefore, the spleen was left on one nerve (Fig. 2).

The typhoid and radioactive vaccines were injected into the spleen connected to the body by only one nerve. The spleen was removed after five minutes, and the wound was sutured under sterile conditions. Seven days after this operation, the titer of antibodies in the animal's blood was determined. A corresponding determination of radioactive phosphorus in the blood of these animals showed that the activity of the blood had changed only very slightly, within the margin for error. In seven days, the titer of antibodies to the typhoid and enteric bacilli in the blood of these animals was determined. The experiments showed that the titer of antibodies to the typhoid bacillus had increased, while the titer of antibodies to the enteric bacillus was practically unchanged (Fig. 3a). Therefore, when an antigen is injected into a spleen connected by one nerve to the body, specific antibodies form in approximately the same quantity as after ligation of the vessels alone. The titer of antibodies to the enteric bacillus changed slightly, which was an indication of the slight, nonspecific change in the antibodies which is caused by the action of any antigen.

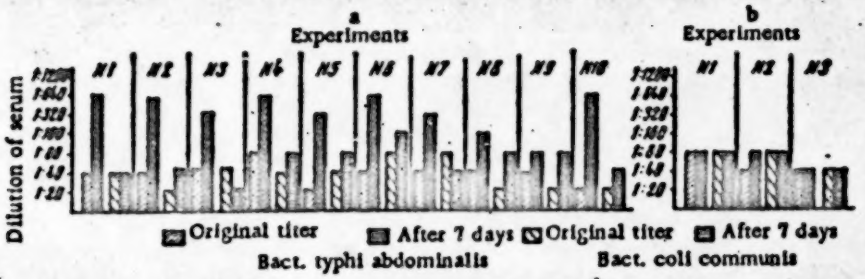

Fig. 1. Changes in the titer of agglutinins in the blood due to typhoid vaccine injection (a) and to physiological solution injection (b – control) into an isolated spleen, followed by splenectomy.

In the third series of experiments, we examined the effect of preliminarily cutting the splenic nerve on the reflex production of antibodies. For this purpose, we isolated the spleen in the experimental dogs by the above-described method and cut the splenic nerve in addition. The typhoid vaccine was injected into the denervated spleen, and additional ligatures were applied to the vessels after 5 minutes; then the vessels were cut between the ligatures, and the spleen was removed. After 7 days, the titer of antibodies in the dogs' blood was determined. In three animals, neither the titer of agglutinins to the typhoid bacillus nor that to the enteric bacillus changed at all; in two animals, it was raised by one dilution (Fig. 3b).

These data make it clear that the antigen acts on the receptors and causes the reflex production of antibodies. Cutting the splenic nerve disturbs the reflex arc from the spleen receptors and also the reflex production of antibodies.

One may assume from the data obtained that this proposed method of splenic isolation makes it possible

Fig. 2. Spleen, with preserved nerve connection and ligated, transected vessels.

to isolate the spleen from circulation and leave it connected with the central nervous system by the intact nerve. Antigen injected into such a spleen, when the spleen is then removed 5 minutes after the injection, causes the production of specific antibodies and does not much change the titer of nonspecific antibodies. Preliminary sectioning of the splenic nerve sharply lowers the production of antibodies to the antigen injected into the isolated spleen. The extirpation operation of the spleen and the manipulations of it associated with the antigen injection do not change the titer of antibodies.

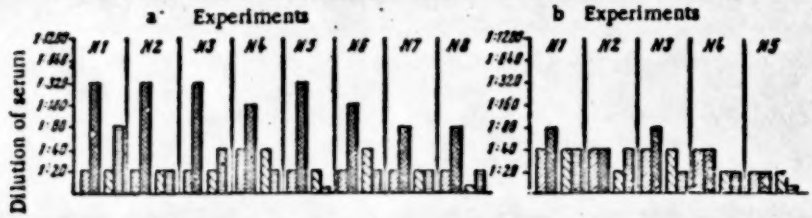

Fig. 3. Change in titer of antibodies caused by injection of typhoid antigen: in isolated spleen with ligated and transected vessels and intact nerves (a) and in denervated spleen (b) with subsequent splenectomy.

SUMMARY

Various antigens were injected into a spleen which had been isolated from circulation. Isolation was effected by separating the splenic nerve and tying all the underlying blood vessels and tissues. The degree of splenic isolation was tested by introducing P^{32} into the spleen and subsequently determining its presence in the blood. When the isolated spleen was removed five minutes after being injected with the antigen, the antigen injection brought about the formation of specific antibodies, although no resorption occurred. When no antigen was injected into the spleen, surgical intervention did not change the antibody titer.

LITERATURE CITED

[1] K. A. Pozdeev, Regarding the Splenic Chemoreceptors' Excitability to Serous and Bacterial Antigens in Animals with Changed and Unchanged Immunological Reactivity," Authors' Abstract of Dissertation, Kazan, 1954.

[2] G. A. Strashun, Zhur. Mikrobiol., Epidemiol. i Immunobiol., 1952, No. 2, pp. 14-17.

[3] E. A. Tatarinov, in the book: Summary of the Reports of the 1st All-Union Conference of Pathophysiologists in Kazan," Moscow 1950, pp. 64-66.

[4] V. A. Tychinin, The Importance of Reception in the Carotid Sinus and Spleen to Immunogenesis and Anaphylaxis Phenomena," Author's Abstract of Dissertation, Kiev, 1950.

" In Russian.

ONCOLOGY

ON THE POSSIBILITY OF THE EXISTENCE OF A SINGLE ANTIGEN SPECIFIC FOR ALL HUMAN CANCERS

V. S. Korostcleva and P. N. Kosyakov

From the Institute of Virusology (Director – Prof. P. N. Kosyakov) Acad. Med. Sci. USSR, Moscow

(Received September 17, 1956. Presented by Active Member Acad. Med. Sci. USSR N. N. Zhukov-Verezhnikov)

It has been established that cancerous growths in man, along with antigens common to both malignant and normal tissues as, for example, the specie [8], group [5, 13, 16], type [6, 7], heterophile [14], contain also specific antigens possessed only by them [2, 9, 11]. These specific antigenic substances have been demonstrated in tumors varying as to localization and structure: malignancies in the liver, stomach, gastrointestinal tract, pancreas, long uterus, ovary, breast and others.

However, the question as to whether all these tumors in varying locations, as well as tumors in one location but of a different origin and in different persons, contain a single antigen, uniquely specific for all human cancers, has not been fully settled.

Using as a basis his experiments with absorbed specific sera, A. K. Saakov [12] concluded that tumors in such varying locations as stomach, uterine cervix and the lung – all possess a general, specific antigen which is common to them all. The same conclusion was reached by Makari and Huch [15] who uncovered the cancer antigen in the serum of patients (28 tumors in different locations were studied) by using the method of active and passive anaphylaxis in guinea-pigs sensitized to the extracts prepared from the isolated organs (Schultz–Delya method).

V. V. Gorodilov and L. V. Shershulsky [1] obtained contrary results. Using as a basis their experiments conducted with anaphylaxis from sensitization by the method of L. A. Silver [2], they concluded that tumors from different organs (cancers of stomach, liver, breast, uterus and ovary) contain not only specific antigens common to them all but also various individual antigenic components. Analogous data were obtained by V. A. Korenevsky [3] when he studied human malignancies in the brain.

The goal of the present study was the further investigation of the similarities or differences in specific cancer antigens present in malignancies growing in various locations.

EXPERIMENTAL METHODS

The material for this study consisted of three malignancies all growing in the same human organ. Tumor Number 1 – metastasis in the liver from a primary adenocarcinoma of the cecum, Number 2 – metastasis to the liver from an adenocarcinoma of the gallbladder and Number 3 – primary liver cancer. The patients having tumors 1 and 2 belonged to the same blood group – O, while tumor 3 was taken from an individual in group B. From each tumor immune sera were obtained from rabbits: Number 388 from tumor 1, Number 528 from tumor 2 and Number 1934 from tumor 3. We have described in detail the methods used to immunize the rabbits and also the methods of purifications used to free the sera from other antigens [9].

The specificity of the antitumor extracts of sera were checked simultaneously against saline extracts of normal human organs – liver and spleen. The latter, as shown in previous experiments [4], has the greatest capacity possessed by any normal human organ for having antigenic similarities with human malignancies and therefore extracts from this organ must always be used in deciding the specific nature of any serum used.

As controls we employed, simultaneously with the antitumor sera, sera specific against the normal human organs (liver, spleen), obtained using the method of N. I. Kuznetsov [10].

As malignancies maintain their antigenic properties after fixation in formalin and glycerin [4], we were able to use, alongside with the antigens of the original tumors, also antigens of preserved tumors.

TABLE 1

Comparative Studies on the Specific Antigenic Properties of Cancers Employing the Complement Fixation Method

Sera	Sera dilutions	Antigens					Control sera
		tumor	tumor	tumor	liver	spleen	
Antitumor number 388 (to tumor 1)	1 : 80 1 : 160 1 : 320	++++ ++++ ++++	– – –	– – –	– – –	– – –	– – –
Antitumor number 528 (to tumor 2)	1 : 40 1 : 80 1 : 160	– – –	++++ ++ ±	– – –	– – –	– – –	– – –
Antitumor number 1934 (to tumor 3)	1 : 20 1 : 40 1 : 80	– – –	– – –	+++ ++ ±	– – –	– – –	– – –
Antiliver 980	1 : 40 1 : 80 1 : 160	– – –	– – –	– – –	++++ +++ ±	– – –	– – –
Antispleen 57	1 : 40 1 : 80 1 : 160	– – –	– – –	– – –	– – –	++++ ++++ +++	– – –
Control antigens		–	–	–	–	–	

Significance of signs used: ++++, +++, ++, +, ± different degrees of positiveness of the reaction; – negative reaction.

EXPERIMENTAL RESULTS

The results of comparative studies on the specific antigenic properties of cancers Numbers 1, 2 and 3 employing the complement binding reaction method is shown on Table 1.

From Table 1 it can be seen that the immune, antitumor serum Number 388 to tumor Number 1, after the removal from it of antibodies to the antigens of normal organs (this goal being achieved by absorbing the serum on spleen extract), loses it ability to react with antigens taken from normal tissues and retains only its capacity to react with water saline extracts of the tumor tissue which had served as the material for immunization. This serum did not react with antigens obtained from tumors Number 2 and 3, this being evidence that any antigen identical with the antigen obtained from tumor Number one must be absent. Similarly, the antitumor serum Number 528, after being treated in the same manner as was serum Number 388, retained only its capacity to react with antigens from tumor Number 2, this being the growth against which this serum was developed. It would not react with antigens obtained from tumors Number 1 and 3. This is taken as proof that these tumors

TABLE 2

Comparative Study of the Specific Antigenic Properties Possessed by Tumors Number 1 and 2 Using the Method of Specific Absorption

Sera	Absorbing tissue	Serum dilution	Antigens: tumor	tumor	liver	spleen	Control sera
Antitumor No. 388 (to tumor No. 1)	Formalinized spleen	1 : 60	++++	−	−	−	−
		1 : 620	++++	−	−	−	−
		1 : 40	++++	−	−	−	−
	Formalinized tumor No. 1	1 : 80	−	−	−	−	−
		1 : 160	−	−	−	−	−
		1 : 320	−	−	−	−	−
	Formalinized tumor No. 2	1 : 80	++++	−	−	−	−
		1 : 160	++++	−	−	−	−
		1 : 320	++++	−	−	−	−
Antitumor No. 528 (to tumor No. 2)	Formalinized spleen	1 : 40	−	++++	−	−	−
		1 : 80	−	+++	−	−	−
		1 : 160	−	+	−	−	−
	Formalinized tumor No. 2	1 : 40	−	++++	−	−	−
		1 : 80	−	+++	−	−	−
		1 : 160	−	+	−	−	−
		1 : 40	−	−	−	−	−
		1 : 80	−	−	−	−	−
		1 : 160	−	−	−	−	−
Antiliver No. 980	Normal organs	1 : 40	−	−	++++	+	−
		1 : 80	−	−	+++	±	−
		1 : 160	−	−	++	−	−
Antispleen No. 980	Normal organs	1 : 40	±	−	+	++++	−
		1 : 80	−	−	−	++++	−
		1 : 160	−	−	−	+++	−
Antigen controls			−	−	−	−	

Significance of the signs as in the Table 1.

do not possess antigens identical with those obtained from tumor Number 2. The antitumor serum Number 1934, after similar removal of antigens against normal organs, maintained only the ability to react with antigens from tumor Number 3, this being the cancer against which this serum was developed. Again, against tumors Numbers 1 and 2 this serum would not react so that we can assume also in this instance that tumors 1 and 2 did not possess antigens identical to tumor Number 3. As indication of the specific nature of the antitumor antigen, the absence

chosen doses of the antigens from normal organs were sufficiently well gauged in preliminary titrations, can be proven by the fact that these antigens reacted positively with the corresponding organ-specific homologous sera Numbers 980 and 57. The sera against the normal organ extract antigens, in their turn, were quite specific and did not react with the antigens taken from the tumors.

In this fashion, our experiments indicate that the tumors we studied which were obtained from three different individuals differ as to their specific antigenic properties. Each tumor has its own, its absolutely specific antigen.

These conclusions are confirmed by experiments with specific absorptions.

The absorption experiment consisted of two phases. The first phase—absorption of the serum by various fixed tissues (cancers preserved in formalin), the second phase—testing the sera upon complement binding by specific antibodies. Before absorption, the pieces preserved in 5% formalin (1 part 40% formalin plus 7 parts water) were washed in continuously running water for 18-20 hours in order that all the formalin would be removed from the tissues, then the material was ground up in a mortar to which physiological saline was added as the tissues were pulverized. The resulting mixture was then centrifuged, the precipitate being repeatedly washed with physiological saline and recentrifuged until finally there was formed only a clear supernatant liquid. Such precipitates from ground tissues washed in this manner with physiological saline were employed in their capacity as absorbents. Each sample of the serum was absorbed by different volumes of the pulverized tissue extracts. The quantity of tissue used for each absorption was related to the titer of the sera being tested, it being possible for it to be different for each individual sample of serum. As a rule, for 1 volume of precipitate there were 4 volumes of serum, diluted 1:10. The pulverized cancer tissues and the serum were thoroughly mixed, the suspension being permitted to stand at room temperature for 30 minutes. Then it was centrifuged for 20 minutes at 4,000 r.p.m., the supernatant liquid was suctioned off and the second phase of the experiment was performed, the complement fixation by the antibodies being measured.

The results of observations made on tumors Numbers 1 and 2 are given in Table 2.

From Table 2 it can be seen that the antitumor serum Number 388, after absorption by tissues of normal organs (spleen) maintained its capacity to react with saline-water extracts from tumor Number 1. This serum did not lose its specific tumor Number 1 antibodies even after being absorbed by tissues from tumor Number 2. When it was allowed to be absorbed by tissues taken from tumor Number 1, this serum lost completely its antitumor titer. Analogous results were obtained by us when we studied serum Number 528. This serum, after absorption by normal organ tissues (spleen), continued to react with saline-water extracts from tumor Number 2, against which it was prepared originally. Extracts from tumor Number 1 could not remove the specific antibodies against tumor Number 2. In contrast, the tissues from tumor Number 2, against which this serum was prepared, completely removed the specific antibodies so that this serum lost all ability to act with its own specific homologous cancer antigen.

The results of these experiments on specific absorption of the antitumor sera by tissue extracts from various tumors confirm the view that these antigens are qualitatively specifically different from each other at least in the cancers studied.

The general antigenic properties of the tumors we studied could be compared with the normal antigenic properties and could be readily differentiated by our use of absorption of immune antitumor sera by use of spleen extract sera from a normal organ.

In this fashion we believe that we have shown quite conclusively that there is no single antigen specific for all human cancers.

Cancers present in the same metastatic locality (liver) can be quite different from each other having qualitatively distinct specific antigenic properties which do not react in the slightest with sera prepared from another tumor.

There must now be explored the further question as to the reason for qualitative differences between the various human cancers arising in different human patients.

SUMMARY

Specific (absorbed) sera against human cancer and tissues of normal organs were used in a comparative study of antigenic properties of human cancerous tumors.

It was demonstrated by the authors' specific absorption method that tumors of the same localization may differ in the character of their antigenic properties. There is no antigen in existence specific for all human cancerous tumors.

LITERATURE CITED

[1] V. V. Gorodilova and A. V. Sherbulskaya, in book: Cancer Pathogenesis and Immunity," Moscow, 1956, pp. 115-127.

[2] L. A. Silver, Uspekhi Sovremennoi Biol., 1949, Vol. 27, pp. 185-211.

[3] V. A. Korenevskaya, in book: Annotated Sci. Studies Acad. Med. Sci. USSR°1954, Moscow, 1955, pp. 261-262.

[4] V. S. Korosteleva, in book: Studies on the Immunity Conditions of Normal and Malignant Tissues," Moscow, 1956, pp. 94-104.

[5] P. N. Kosyakov, Zhur. Mikrobiol., Epidemiol. i Immunobiol. 1936, Vol. 16, No. 2, pp. 215-232.

[6] P. N. Kosyakov, Byull. Eksptl. Biol. i Med., 1952, No. 2, pp. 54-56.

[7] P. N. Kosyakov, Byull. Eksptl. Biol. i Med., 1952, No. 3, pp. 57-59.

[8] P. N. Kosyakov, Byull. Eksptl. Biol. i Med., 1952, No. 9, pp. 62-64.

[9] P. N. Kosyakov, V. S. Korosteleva and N. I. Kuznetsova, Byull. Eksptl. Biol. i Med., 1955, No. 9, pp. 63-69.

[10] N. I. Kuznetsova, Byull. Eksptl. Biol. i Med., 1955, No. 11, pp. 52-55.

[11] A. K. Saakov, Byull. Eksptl. Biol. i Med., No. 8, pp. 61-62.

[12] A. K. Saakov, Byull. Eksptl. Biol. i Med., No. 11, Vol. 38, p. 53.

[13] M. Goldstein and E. Kreisel, Boll. Inst. Sierotherap. Milanese, 1930, Vol. 9, p. 105-108.

[14] H. Lehmann-Facius, Ztschr. f. Immunforsch., 1928, Bd. 56, S. 464-515.

[15] I. G. Makari and M. G. Hut British medical Journal, 1955, N. 26, 1291-1995.

[16] O. Thomsen, Acta path. et microbiol. Scandinav., 1930, Vol. 7, 250-257.

THE EFFECT OF A LIVE CULTURE OF B. PRODIGIOSUS ON TRANSPLANTED TUMORS IN EXPERIMENTAL ANIMALS

N. I. Rybakov

From the Division of Immunology (Chief Active Member of the Academy of Medical Sciences USSR, Prof. N. N. Zhukov-Verezhnikov) Institute for Experimental Biology (Director – Prof. I. N. Maisky) Academy of Medical Sciences, USSR, Moscow

(Received December 28, 1956. Presented by Acad. Med. Sci. Prof. N. N. Zhukov-Verezhnikov)

As early as the last century, attempts were made to treat malignant new growths by the utilization of microorganisms and their products. N. F. Gamaleya [1,3] was the first to propose this toxitherapy of cancer in the environment of the oncological clinic, utilizing for his goal the toxins of a killed culture of B. prodigiosus.

Later many investigators [2,4,6,7,8 and others] studied the influence the toxins of this microbe had upon various cancers, both in the clinic and experimentally. Many of them [4,5,6] succeeded in suppressing the growth of experimental tumors in animals by introducing into them the toxins from B. prodigiosus cultures; there being seen a picture of hemorrhages into the tissues of the growth itself.

The next stage in the study of this problem consisted in attempts to fractionate the cultures of this microbe in such a manner as to isolate that B. prodigiosus fraction which was active against the cancers [9,10]. Still, the whole question of the therapeutic effectiveness of B. prodigiosus cultures and its various fractions has remained open.

In developing experimental methods of complex bio-immunological therapy of malignant new growths, we set ourselves the task of investigating the influence exerted by a living culture of B. prodigiosus upon tumor transplants, inasmuch as we are aware of only a single study devoted to this question [4].

METHODS AND RESULTS

In these experiments we used white rats, males weighing 250-280 g and male chinchilla breed rabbits weighing 2,5-3 kg each.

M-1 sarcoma was injected subcutaneously into the rats in the form of a 10% suspension, the dose being 0,25 ml; 20% suspension of Brown-Pierce tumor was injected intratesticularly to the rabbits, the dose being 0,5 ml. Among the factors needing consideration as to the effect of living cultures of B. prodigiosus upon tumor growth, was the obtaining by us of three fresh strains (Numbers 1,8 and 10) of living B. prodigiosus from the L. A. Tarasevich Central State Institute of Scientific Standards. We conducted preliminary titrations with these cultures as to their toxicity.

Experiments proved that a single intraperitoneal injection, into rats of 400 million microbes per 1000 g of body weight and, into rabbits, of 1,5 billion microbe bodies per 1,000 g of body weight, had but little effect upon the animals. If the injections were repeated at 3 day intervals, both the rats and the rabbits did lose a little weight but otherwise withstood the inoculations with B. prodigiosus quite well. The rats had less of a reaction than the rabbits which would develop a fever, lose their appetite and not return to a normal state for

2-3 days. The first experiments were performed on rats for a comparative study of the influence exerted by the three living strains of B. prodigiosus on sarcoma M-1. 50 rats, all of about the same age and weight, were used in the first experimental series. Commencing with the 9th day after the subcutaneous inoculation of tumor M-1, three animal groups (10 in each) received intraperitoneally the corresponding strain of B. prodigiosus.

As controls, we had an animal group which received intraperitoneally saline solution only. The size of the tumor was measured by using the formula for the area of an ellipse. After giving 5 injections at 3 day intervals, i.e., on the 15th day after beginning the injections, the rats were sacrificed and the tumors excised and weighed.

In further experiments we examined the influence exerted by a living B. prodigiosus culture, strain Number 10, upon the capacity of the Brown-Pierce tumor to metastasize. 11 rabbits were used, 6 being injected and 5 serving as controls. On the 9th day following the intratesticular introduction of the tumor suspension, the rabbits received intravenously 700 million microbe bodies of this culture per 1 kg body weight. After that, the injections were given intraperitoneally at 3 day intervals. A total of 7 injections was given.

The results of the experiments conducted on the rats in order to observe the influence of living B. prodigiosus cultures on sarcoma M-1 are shown in Table 1.

TABLE 1

Influence of Living Culture of B. Prodigiosus (by intraperitoneal injection) Upon the Development of Sarcoma M-1

Strain	Number of animals		Average dimension of tumor (in cm^2)		Average weight of tumor after sacrificing the animals (in mg)
	used in exp.	perished during experiment	before injection	at end of exp.	
1	10	—	1.3	3.2	7.467
8	10	3	1 7	6.1	21.365
10	10	—	1.1	2.8	4.089
Control	20	1	1 0	11 5	28.619

From Table 1 it can be seen that the living culture of B. prodigiosus does have a somewhat inhibitory influence upon sarcoma M-1 growth, this being most marked when strain Number 10 was used. Analogous results were seen in the rats who received subcutaneous injections of strain Number 10 B. prodigiosus in the vicinity of the growing sarcoma M-1 (Table 2).

However, it should be stated that, in these experiments, we did not observe complete regression of the tumor as a result of the microbe having been introduced.

Microscopic studies of the tumors removed from the rats receiving B. prodigiosus injections, revealed large zones of tumor necrosis extending from the center to the periphery of the growth, there being seen, along with well growing areas of malignancy, masses of degenerating cancer cells having markedly abnormal nuclear structures. Quite often, even in the healthy cancer areas, there could be seen extensive focal hemorrhages on the edges of which there could be seen beginning necrosis being walled-off by a leucocytic barrier. In individual instances, the hemorrhages would involve the greater portion of the tumor capsule. Such microscopic observations could be made in the tumors of the control animals.

The influence exerted by live cultures of B. prodigiosus upon the malignant Brown-Pierce tumors is seen when Table 3 is examined.

Table 3 shows that, of the 6 experimental animals there was full tumor recession in 4 rabbits, the testicular growth dissolving completely. These rabbits stayed well for 8 months after being inoculated with the Brown-Pierce tumor. The remaining rabbits of this group perished at various times from the beginning of the experiment. Rabbit Number 2298 died on the 32nd day after being inoculated with the tumor as a result of

TABLE 2

Animal group	Animals		Average dimension of tumor (in cm^3)		Average weight of tumor after sacrificing the animals (in mg)
	used in exp.	dying during exp.	before injection	at end of experiment	
Experiment	6	—	1.5	3.8	9.042
Control	6	—	1.4	15	38.750

multiple metastasis. These were especially numerous in the epiploic lymphatics, in the loops of the large and small intestine and in the mesentery. Rabbit Number 1095 died 150 days after being inoculated with the Brown-Pierce tumor. At autopsy, there was found a 5×3 cm tumor in the side of the left seminal cord. This cancer metastasis was an enormous necrotic mass with massive liquefaction of the tumor tissue itself. It should be noted further that this rabbit had areas of focal inflammation in the central portions of both lungs there being pronounced carnification of pulmonary tissues. All the control animals died on the 30-32nd day after being injected with the Brown-Pierce tumor.

TABLE 3

Animal group of rabbits	Used in exp.	Tumor took	Dying during exp. period by 32d day	Tumor dissolved	Surviving to 8th month and longer
Experiment	6	6	1	4	4
Control	5	5	5	—	—

Summarizing, we can state that living cultures of B. prodigiosus exert a varying suppressing effect upon rat sarcoma M-1, the different strains of the microbe exhibiting variations in their effectiveness. The antitumor effect of the living microbe is seen very strikingly in the fact that the Brown-Pierce tumor was seen to fully regress and dissolve in the majority of animals.

SUMMARY

Intraperitoneal inoculation of three different strains of live cultures of B. prodigiosus led to a varying regression of rat sarcoma M-1 injected subcutaneously. In rabbits inoculated intratesticularly with a Brown-Pierce tumor, there was observed a complete destruction of the malignancy, the large majority of the animals surviving for 8 months and more.

LITERATURE CITED

[1] N. F. Gamaleya, Foundations of Immunology. Studies on Incompatibility and Vaccination, Moscow–Leningrad, 1928, p. 103.

[2] S. P. Zaeva, in book: Clinical Treatment of Tumors,* Riga, 1953, Vol. 1, pp. 117-133.

[3] L. I. Korenevsky, Vrach. Delo, 1954, Issue 4, pp. 361-362.

[4] M. Ya. Sukhin, Doklady Akad Nauk USSR, 1941, Vol. 32, No. 9, pp. 670-673.

* In Russian.

[5] G. Algire, F. Legallais and B. J. Anderson, Journ. Nat. Cancer Inst., 1952, Vol. 12, No. 6, pp. 1279-1295.

[6] S. P. Beebe and M. Tracy, Journ. Am. Med. Assoc., 1907, Vol. 49, pp. 1493-1498.

[7] W. B. Cooley, Trans. Am. Surg. Assoc. Philadelphia, 1894, Vol. 12, pp. 183-212.

[8] W. B. Cooley, Trans. Am. Surg. Assoc. Philadelphia, 1932, Vol. 50, pp. 383-417.

[9] M. L. Shear and F. C. Turner, J. Nat. Cancer Inst., 1943, Vol. 4, pp. 81-97.

[10] M. L. Shear, J. Nat. Cancer Inst., 1944, Vol. 4, pp. 461-476.

GROWTH OF THE EHRLICH ADENOCARCINOMA WHEN TWO TUMORS ARE TRANSPLANTED SIMULTANEOUSLY OR AT DIFFERENT TIMES INTO VARIOUS REGIONS OF THE ORGANISM

O. I. Epifanova and I. N. Smolenskaya

From the histologic laboratory of the Institute for Experimental Biology (Chief – Candidate in Biological Sciences V. N. Dobrokhotov) (Director – Prof. I. N. Maisky) Academy of Medical Sciences USSR Moscow

(Received July 3, 1956. Presented by Active Member of the Academy of Medical Sciences USSR Prof. N. N. Zhukov-Verezhnikov)

In a preceding study [2] it was noted that the rate of growth of an Ehrlich carcinoma had a relation to the site of inoculation. It was proven that the adenocarcinoma inoculated into the anterior limb of a mouse grew faster than a similar tumor injected into a posterior limb. At that time there was not demonstrated any regulating factor controlling growth rate of the adenocarcinoma within the limits of one body segment.

The aim of the present study was to observe whether these relationships are preserved when two adenocarcinomas are inoculated into the animal.

EXPERIMENTAL METHODS

300 mice were used–sexually mature females averaging in weight about 20 gm. To produce the subcutaneous tumors, we used, as before, a suspension of the Ehrlich adenocarcinoma (0.2 ml with each injection). In some of the experiments the two tumors were injected simultaneously into different regions of the body, the weight of the tumors being determined when the animals were sacrificed on the 15th day. In some of the other experiments the interval between the two inoculations consisted of 10 days, the animals being sacrificed 10 days after the second inoculation. In each series there was a control group which received only the one inoculation into the corresponding portion of the body. When comparing the rate of growth for the tumors, the probabilities for their attaining a given size were calculated mathematically from the average of their weights P [4].

EXPERIMENTAL RESULTS

Figure 1 presents the data on the average weights of the tumors inoculated simultaneously into different body regions of the mouse, the number of animals used in each group being indicated within the parentheses.

Analysis of the figure indicates that simultaneous inoculation of two Ehrlich adenocarcinomas results in the one inoculated into the region of the fore limb outgrowing the one inoculated into the region of the hind limb on the same side ($p = 0.021$). Within the limits of the one body segment there is no difference in the rate of growth for the two simultaneously inoculated tumors ($p = 0.368$ and $p = 0.484$).

Figure 2 presents the averages for the tumors simultaneously inoculated into the fore limb on one side and the hind limb on the other. In this situation, also, the tumor on the fore limb grows more rapidly than the one in the hind limb (in control, $p < 0.0001$; in experiment, $p = 0.0001$). The general rate of growth is much greater here than in the preceding series. At the same time, simultaneous inoculation of the two tumors never caused the average tumor weights to decrease when compared with the controls.

Thus it can be seen that the peculiarities in the growth of Ehrlich adenocarcinomas arising from the region

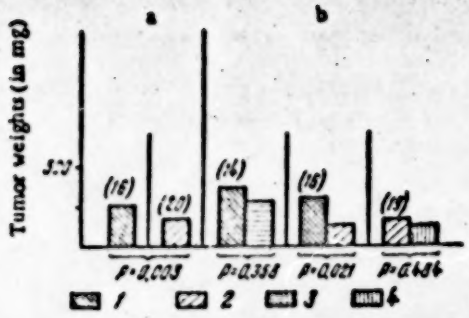

Fig. 1. Growth of Ehrlich adenocarcinoma in the region of the fore limbs and hind limbs on the same side of the body when the inoculations were made simultaneously and the animals were sacrificed 15 days after the inoculations. a) experiment; b) control; 1) right fore limb; 2) right hind limb; 3) left fore limb; 4) left hind. Within parentheses is indicated the number of animals.

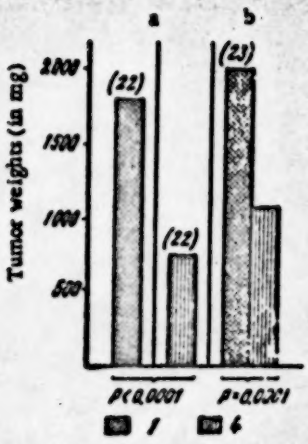

Fig. 2. Growth of Ehrlich adenocarcinoma simultaneously injected into the region of one fore limb and the region of the hind limb on the opposite side, the animals being sacrificed 15 days after inoculation. Labelling as in Figure 1.

of the inoculation sites manifest themselves also when two tumors are inoculated into the animal simultaneously.

In Figure 3 are shown the average tumor weights of 20-day growths injected into the right fore and hind limb regions. The experimental animals received, 10 days after the first inoculation, a second tumor inoculation in the region of the other limb of the same side.

There was no significant difference in the rate of growth on the part of the first inoculated tumors ($p = 0.368$). At the same time, in the control animals, the average weight of the tumors in the anterior regions was greater than in the posterior ($p = 0.0004$).

When there was inoculated an adenocarcinoma into animals already having a 20 day tumor, there did not develop a difference in the growth of 10 day tumors when those in the region of the fore limb were compared with those in the region of the hind limb, neither in the experiment ($p = 0.424$) nor in the control ($p = 0.134$). It was not clear why the control animals failed to show a difference.

This does seem to show, however, that, when two Ehrlich adenocarcinomas are inoculated into the same animal at different times, the peculiarities in the rates of growth previously noted were no longer present.

The average weight of the 10-day tumors in the control groups exceeded the weight of the similar carcinomas inoculated into corresponding areas into animals already having tumors ($p = 0.0001$ and 0.021).

This seems to indicate that the presence of a previous tumor gives a certain degree of immunity to a repeated inoculation with the same tumor.

In one case, there were no differences (see Fig. 3) in the growth of the 20-day tumors and the controls ($p = 0.23$), in another instance the average weight of the 20 day tumors in the controls exceeded the weights in the corresponding group ($p = 0.0003$). This last speaks against the prevailing view that the organism develops resistance as the secondary tumors develop, not interfering with the growth of the original inoculations [1].

It is possible that we should seek in this the reason for the fact that there is no difference in the rate of growth of the 20 day tumors as far as the tumors in the anterior and posterior limb regions are concerned.

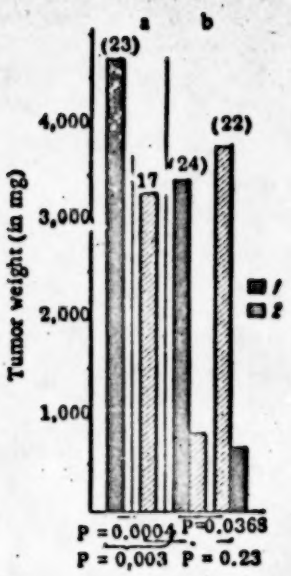

Fig. 3. Growth of Ehrlich adenocarcinoma in the fore and hind limb regions on the 20th day when there is already present a 10-day tumor. Labelling as in Fig. 1.

The underlying reasons for the uneven rates of adenocarcinoma growth depending on the sites of inoculation have not been analyzed so far. We have shown in the present study that secondary inoculation upsets this differential. This second inoculation can be regarded as an additional stimulant [3] which, with the aid of certain reflex mechanisms, upsets the rate of growth for the first tumor inoculated, altering the resistance in the focus of its development. However, we cannot talk with assurance about the nature of these reflex mechanisms altering the organism resistance by virtue of the second tumor having been inoculated, until after additional studies have been made, namely, inoculating the secondary irritant (second tumor) into various regions of the body.

As long as we do not possess this additional data, we can only draw attention to the single fact that secondary inoculation of the adenocarcinoma causes such immunological changes within the organism that the growth differentials previously noted are no longer present.

SUMMARY

When two Ehrlich adenocarcinomas were transplanted simultaneously, the anterior one grew more intensely. When there was time interval between the two inoculations, this difference disappeared, probably as a result of immunological alterations arising in the organism.

LITERATURE CITED

[1] F. M. Bricker and L. I. Tinofeeva, in book: Antitumor Immunity,* pp. 9-19 (1939).

[2] O. I. Epifanova, Byull. Eksptl. Biol. i Med., 1956, No. 11, pp. 55-57.

[3] S. I. Lebedinskaya and A. A. Soloviev, Klin. med., 1951, Vol. 29, No. 3, pp. 11-15.

[4] V. I. Romanovsky, Use of Mathematics in Statistical Evaluation of Experimental Data,* Moscow–Leningrad (1947).

* In Russian.

EXPERIMENTAL BIOLOGY

CRANIAL BONE REGENERATION EFFECTED BY TRANSPLANTATION OF GROUND MAMMAL BONES

L. V. Polezhaev, A. I. Matveeva and N. A. Zakharova

From the A. N. Severtsov Institute of Animal Morphology (Director – Prof. G. K. Khrushchov, Corresponding Member of the USSR Academy of Sciences), USSR Academy of Sciences, Moscow

(Received April 4, 1956. Presented by Prof. O. B. Lepeshinsky, Active Member of the USSR Academy of Sciences)

Surgeons usually employ alloplasty, i.e., transplantation of nonliving plates (metal, horn, plastic) to replace defects in nonregenerating, integumentary, human cranial bones. However, foreign material cannot avoid causing some harmful effect on healthy living tissues and organs – for example, the brain; therefore biological methods for covering defects with living bony tissue should be developed. Autoplastic or homoplastic transplantation of developed bone or cartilage is difficult and is attended by the resorption of the transplantates. In previous works [8,9,10], we proposed three biological methods, one of which was used successfully in another laboratory [2,3].

In this work, we propose a new, simpler method and also present data regarding the dependency of mammalian integumentary cranial bones' regeneration on age.

When setting up the experiments, we started from the following premises. In cases where a defect is artificially induced in the integumentary cranial bones of mice, dogs and other animals, although regeneration over the actual edge of the undecayed bone in the periosteum is absent, slight signs of osteogenesis are nevertheless evident [10]. Organs regenerate more efficiently and completely when their component tissues are destroyed [9]. The tails and extremities of mature axolotls can regenerate when their tissues are completely destroyed [8]. A. N. Studitsky [12] also obtained complete regeneration after destroying muscles capable of regeneration in roosters and rats. We proposed that slightly decaying mammalian integumentary cranial bones, nonregenerating under ordinary conditions, can be stimulated to regeneration by means of artificial intensification of their decay by destruction.

EXPERIMENTAL METHODS

A total of 54 animals were used in the experiment. Destruction experiments were conducted on 11 adult white mice and 11 white rats, and experiments to determine the dependency of regeneration on age, on 4 five-day old kittens and on 8 eight-day old rats. Besides this, a cranial roof defect was artificially induced in 10 adult mice and 10 adult rats, which were used as the control. The operation was performed under sterile conditions. After ether anesthesia had been administered and the fur had been plucked and shaved, an incision was made into the skin of the head to the left of the center line. Rectangular pieces, constituting about half of the surface of the parietal bones, were extirpated from the cranium on the right; the pieces were 6×3 mm in the mice, 8×5 mm in the rats and 20×15 mm in the kittens. In the rats and kittens, the bone was sawed out with a special saw, but a scalpel was used for the operation on the mice. Special care was taken to prevent damage to the cerebral membranes, although they were slit in one or two places in a few cases. In the destruction experiments on the mice and rats, the sectioned cranial roofs were ground into a powder on a cutting grinder of V. E. Sokolov's design, the bone dust was moistened with a solution of penicillin prepared in a physiological solution, and the resulting thick paste was transplanted homoplastically in a thick layer on to the region of the

cranial defect, partially covering the edge of the old bone. After the operation, the region of the wound was drenched with penicillin, the skin was sutured, the wounds were covered with streptocide, and the animals were injected with penicillin in doses of 2,000 to 10,000 units for a period of 2-5 days. All the animals lived. In the destruction experiments, the mice were sacrificed on the 10th, 16th, 60th, 84th and 120th days, and the rats, on the 23rd, 80th and 150th days after the operation. In the experiments examining the age factor, the kittens were killed on the 34th, 60th and 96th days, the rats on the 60th, 102nd and 124th days after the operation. pieces were fixed in 10% formalin, Zenker's fluid with added formalin or acetic acid, decalcined in 5% nitric acid, imbedded in celloidin-paraffin, cut into lateral sections 10 microns thick and stained by Mallory's method or with hematoxylin-eosin.

EXPERIMENTAL RESULTS

In the destruction experiments, unfortunately, the paste sometimes fell out of the defect region while the skin was being sutured. When it was displaced, a thin, supple, fibrous layer appeared in the region of the defect, just as in the control, with the artifical inducement of a simple defect. In the places where the paste remained in place, the cranial defect was completely or partially filled with newly formed, regenerated bone. The regeneration process of the cranial bone differed somewhat in the mice and rats. In both, a reddening of the skin and a severe inflammation of the tissue in the region of the wound was observed the first days after the operation. This abated on the 10th day in the mice; in the region of the defect, between the edges of the cranial bone, a thick layer of immature connective tissue formed, made up of small and somewhat larger bone particles, distributed at random, which became oriented along the line of tension (Fig. 1). The cells had disappeared in almost all of the particles, and the bony substance was partially dissolved. Such a picture was ob-

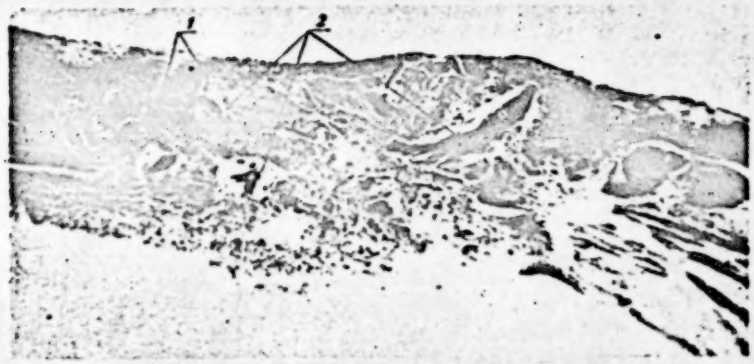

Fig. 1. Destroyed tissue in the region of the defect in a parietal bone of a mouse on the 10th day after the operation.
1) connective tissue; 2) bone particles.

served in both the central and peripheral parts of the transplanted mass, where the particles lay on the edge of the old bone. On the 16th day after the operation, the connective tissue and the whole destroyed mass began to thicken. On the 80th day and 120th day, the destroyed mass became firm and hard, like normal cranial bone, and consisted of newly formed, coarse-fibered bone, in which many acellular bone particles appeared, sealed into it, colored like normal bone and showing no signs of necrosis. The newly formed bone was especially thick and dense in the parts adjoining the old bone (Fig. 2). It had no bone marrow cavities and was considerably younger than the old bone, which had a laminated, dense structure with many bone marrow cavities. The edges of the old bone adjoining the destroyed mass and the newly formed bone were somewhat more rejuvenated than the more distant portions of the old bone. Many bone cells appeared in the bone particles lying on the old bone; the particles were enveloped in newly formed, dense bone, which also contained osteocytes, and were organically fused with the surrounding bony tissue. The periosteum on the old bone, where the bone particles lay, disappeared and was changed into newly formed bone. A new periosteum, covering the upper surface of the newly formed bone, which had replaced the destructive mass, formed from connective tissue.

The picture was similar to that observed by T. P. Vinogradova [4] in experiments establishing that, in plastic operations performed on humans, pieces of hyaline cartilage stimulate the surrounding connective tissue to metamorphose into a periosteum. In cases where the transplanted tissue had either fallen out or been completely resorbed, i.e., when it was not retained, new, thick, coarse-fibered bone nevertheless formed in the region of the defect, but its calcification was somewhat retarded.

Fig. 2. Dense, newly-formed bone in the region of a cranial defect in a mouse on the 80th day after transplantation of the destroyed bone.

In the rats, the destroyed mass was fully resorbed by the 23rd day after the operation, and, at the same time, new young bone formed in the middle of the layer while regeneration occurred from the edges of the old bone. On the 84th and 150th days after the operation, the newly formed bone looked like well-developed laminae, connected with the edges of the old bone and like small plates distributed over the rest of the defect surface. Histological study showed that the regenerating bone was thinner and younger than the old cranial bone.

In the rats, the destroyed bone tissue was not incorporated into the regenerate, but stimulated the surrounding connective tissue to regenerative, new formation just as cartilage, or extracts from it, stimulate connective tissue to become cartilage [7, 13]. In the mice, the destroyed tissue was itself incorporated into the newly formed bone besides, and took part in its construction. Was this tissue living? Evidently it was, since many bone particles in the mice contained live bone cells, but even if there were no live bone cells, their basic bone substance would nevertheless have been preserved and would have stained the same as normal bone. This question will be resolved by special study.

The special feature of this work was the use of the destruction method to obtain regeneration of the integumentary cranial bones in several kinds of adult mammals, which ordinarily are nonregenerating, and obtained a positive result. A similar method has been used by surgeons successfully on tubular bones [6].

This simple and effective method of cranial bone destruction must be perfected on dogs, after which, it may be considered for clinical use. It does not cause inflammatory phenomena or commissures in the cerebral membranes or in the brain.

In the experiments examining the influence of the age factor on the regeneration of the integumentary cranial bones, complete regeneration occurred in all of the kittens after the cranial bone piece had been removed (Fig. 3). Regeneration was most intense along the course of the large blood vessels lying in the grooves between the convolutions of the cortex of the cerebral hemispheres, then spread over the entire surface of the dura mater in the region of the defect. On the 34th day after the operation, the connective tissue stage of cranial development was reached, and approximately one-third of the regenerate surface was filled with young, newly-formed bone. On the 60th day, ossification had extended over the entire surface of the defect, and on the 96th day, the still-developing bone had become very thick. The bone regenerated by new formation, similar to bone development in normal embryogenesis. The edges of the thick, old bone were noticed with numerous bone marrow lacunae and sharply delimited from the regenerating bone, although both bones were indirectly

joined in many places with connective tissues which had not yet ossified. The shape of the regenerated bone followed that of the brain – the ridges and the prominences on the inside surface of the regenerated cranial bones always conformed exactly and strictly to the surface of the underlying brain and vessels lying in the grooves between the brain convolutions. In one case where, due to intracranial pressure, the brain with the membranes protruded like a knob into the bone defect, the bone regenerate formed exactly the same prominence.

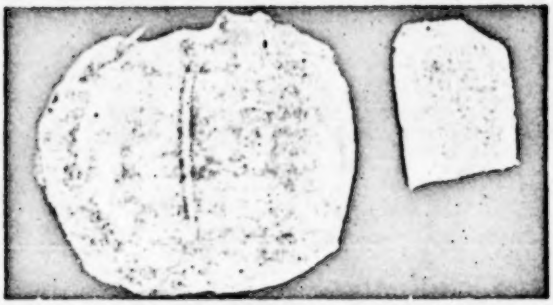

Fig. 3. Complete cranial bone regneration in a kitten on the 96th day after removal of the bone piece, which is shown next to cranium. Seen from below.

In the experiments on the baby rats which lasted up to 124 days, the cranial bone only regenerated in 2 cases; in 6 cases, there was no regeneration, but the defect was overgrown with a supple fibrous layer. The regenerated bone was not uniform in thickness; it was thick at the edges of the old bone and thin in other places, but sometimes formed thick plates. In our preceding experiments, it was shown that the cranial bones can regenerate completely in 26 days in puppies a month old [1,5,8,9,10,11]. The present data show that complete regeneration of the cranial roof bones can also occur in 5-day old kittens, although more slowly than in the dogs. The cranial bones do not regenerate well in 8-day old baby rats (2 positive cases versus 6 negative cases), but can regenerate. The cranial bones do not regenerate in adult dogs, rats and mice, as we were able to conclude earlier. Consequently, the example afforded us by the cranial bones of mammals confirms one of the principal rules of regeneration, i.e., that the ability to regenerate decreases as the animal grows older. Cranial bone regeneration in the destruction experiments was similar to that occurring in young animals, which have the ability to regenerate a given organ.

Therefore, homoplastic transplantation of freshly-destroyed material obtained from the integumentary cranial bones causes the bone in the region of the cranial bone defect to be completely restored in adult mice and rats, whose integumentary bones cannot regenerate.

The regeneration of newly-formed bone is effected by the influence of the destroyed material on the connected tissue, which it stimulates to become bone (in rats). In mice, the destroyed material also participates directly in the formation of the new bone.

Unlike adult animals, which cannot regenerate the integumentary bones of the cranium, young animals, 5-day old kittens and 8-day old baby rats, like one-month old puppies, can regenerate these organs completely. Regeneration of the integumentary cranial bones proceeds similarly to the new formation of bone which occurs in embryogenesis.

Cranial bone regeneration artificially effected by the destruction method is similar to that occurring after simple extirpation of a cranial bone piece in young animals.

SUMMARY

In order to stimulate regenerative bone formation in adult mammals experiments were conducted in replacing cranial bone defects by ground cranial bone. In rats the homotransplanted bone grindings resolved in

three weeks. In mice – more slowly – they werepreserved for several months. Bone grindings stimulated transformation of local connective tissue into genuine bone tissue, and replaced cranial bone defects both in rats and mice.

LITERATURE CITED

[1] N. F. Barakina, G. L. Gintsburg and others, Doklady Akad. Nauk SSSR, 1952, Vol. 87, No. 4, pp. 673-675.

[2] A. N. Brudastov, Doklady Akad. Nauk SSSR, Vol. 86, No. 5, pp. 1057-1060.

[3] A. N. Brudastov, Doklady Akad. Nauk SSSR, 1954, Vol. 94, No. 1, pp. 161-164.

[4] T. P. Vinogradov, Transplantation of Cartilage in Humans (pathologico-histological studies on autohemoplastic data),* Moscow, 1950.

[5] G. L. Gintsburg, Doklady Akad. Nauk SSSR, 1952, Vol. 87, No. 5, pp. 869-872.

[6] Z. L. Kartashev, Tubular Bone Regeneration from Transplanted Small Bone Pieces and Ground Bone (experimental study),* Rostov-on-Don (1930).

[7] N. V. Nasonov, Doklady Akad. Nauk SSSR, 1934, Vol. 3, No. 3, pp. 202-204.

[8] L. V. Polezhaev, Arkh. Anat. Gistol. i Embriol., 1934, Vol. 13, No. 1, pp. 91-109.

[9] L. V. Polezhaev, in the book: Works of the Institute of Cytology, Histology and Embryology,* 1948, Vol. 2, pp. 1-130.

[10] L. V. Polezhaev, Doklady Akad. Nauk SSSR, 1951, Vol. 77, No. 3, pp. 525-528.

[11] L. G. Rogal, Doklady Akad. Nauk SSSR, Vol. 83, No. 6, pp. 953-956.

[12] A. Studitsky, in the book: Problems of Organic and Tissue Regeneration in Vertebrate Animals,* Moscow, 1954, pp. 225-295.

[13] A. Fischer, Arch. f. Entw. mech. d. Organ., 1931, Bd. 125, H. 2-3, S. 203-209.

* In Russian.

THE EFFECT OF LIVER TISSUE HOMOGENATE ON GROWTH IN THE LIVER OF TADPOLES

L. K. Ramanova

From the Laboratory of Growth and Development (Head – Prof. L. D. Liozner), the Institute of Experimental Biology (Director – Prof. I. N. Maisky), Acad. Med. Sci. USSR

(Received September 21, 1956. Presented by Prof. N. N. Zhukov-Verezhnikov, Active Member of the Academy of Medical Sciences USSR)

Specific growth stimulation in the organs of chicken embryos (liver, kidneys, heart) has been obtained by many researchers [1,3,4,5,9] by the administration of a water-salt extract made from the tissue of the same organs. Some authors [3,5,8] propose that substances are formed in the living cells which specifically affect the processes of growth and tissue differentiation and the presence of autoregulation of organic growth and development due to their action [3,8].

According to H. Teir [7], the tissue suspension contain substances which stimulate mitoses and influence cell differentiation. McJukin and Breuhaus [6] observed mitoses to increase in the liver of mice after partial hepatectomy, with the intraperitoneal injection of liver tissue.

The only work which has studied the problem of specific stimulation of the regenerating process in the liver of amphibians with the injection of an extract from the same is not convincing [2]; it lacks reliable criteria by which to judge the regeneration of the liver.

The purpose of this work was to ascertain how widely the rule regarding organic growth stimulation effected by extracts from tissue of corresponding organs applies, and how specific the effect of the extracts is. The relation of the specific effect to the age of the donor of the organ from which the homogenate was prepared and to the age of the recipient was also studied.

EXPERIMENTAL METHODS

The studies were done on Rana temporaria and Rana ridibunda tadpoles.

The animals were divided into 2 groups. The first group (4 series of experiments) contained 197 tadpoles, and 157 tadpoles were used as the control.

The second group (also 4 series of experiments) contained 51 tadpoles, and 42 tadpoles were used as the control.

In the experiments on the first group, R. temporaria and R. ridibunda tadpoles, at the I, II, and III stages of metamorphosis (according to L. Ya. Blyakher) were intraperitoneally injected with a suspension of liver tissue from R. temporaria at stage V.

In the experiments of the second group, R. ridibunda tadpoles, at stages II and III, were intraperitoneally injected with liver tissue from the same species of tadpole at the 1st or an even earlier stage of metamorphosis, before digital differentiation had occurred.

The liver tissue suspension was prepared immediately before the injection. The liver tissue was ground

in a quartz mortar together with a 0,75% solution of NaCl, using a wooden pestle, to a homogeneous mass (ratio by weight of liver tissue to NaCl solution – 1:2).

The animals of the first group were injected once with 8-10 mg of the liver tissue homogenate, the animals of the second group, with 10-15 mg. The control tadpoles were injected intraperitoneally with the same amounts of a 0,75% solution of NaCl. After 3-6 days, the tadpoles were killed, and the entire body of the animal, the liver, pancreas and anterior extremity were weighed.

EXPERIMENTAL RESULTS

In the experiments of the first group, the liver tissue suspension from the R. temporaria stage V tadpoles specifically stimulated growth in the liver of the R. temporaria and the R. ridibunda Stage I, II and III tadpoles; the relative weight increase of the liver – average of each series – was 0,12%, 0,28%, 0,32% and 0,19%. When the material was statistically processed according to Styudent's formula, corresponding values of t were obtained: 2,4, 2,3, 4,5, 3,8, i.e., the changes observed were proven statistically (Table 1).

TABLE 1

Change in Relative Weight of Liver, Pancreas and Anterior Extremity in R. temporaria and R. ridibunda Tadpoles Caused by Injection of Liver Homgenate from R. temporaria Stage V, Tadpoles

Animal group	Recipient	Stage	Duration of experiment in days	Number of tadpoles	Relative weight			t, of Styudent's formula for relative wt., in exp. and control	P, according to Styudent's tables
					of liver	of pancreas	of anterior extremity		
Experiment	R. temporaria	I—II	3	70	0.93	—	—	2.4	0.0164
Control				43	0.81	—	—		
Experiment	The same	III	3	50	2.61	0.270	0.460	2.3	0.0214
Control				49	2.33	0.260	0.410		
Experiment	R. ridibunda	II	3	18	1.48	0.420	0 059	4.5	0.0000
Control				19	1.16	0.390	0.057		
Experiment	The same	0—I	4—5	59	1.41	0.390	0.045	3.8	0.0001
Control				46	1.22	0.400	0.058		

However, there was no statistically verified increase in the weight of the pancreas or anterior extremity in one of the first group series. In two series, the average increase of relative weight of the pancreas was 0,01% and 0,03%, but in another series, the relative weight of the pancreas, as compared with that in the control animals, even decreased (experiment – 0,39%, control – 0,40%).

The stimulating effect of the liver homogenate was observed even 5 days after its injection. For example, in one series of experiments, the average relative weight of the liver after 5 days was 1,41%, as opposed to 1,22% in the control; t in Styudent's formula was 3,8, i.e., the difference between the experiment and the control was not accidental.

In the second group of experiments, the relative weight of the liver tended to decrease (Table 2).

In three series, the average decrease of relative liver weight in the experiment as compared with the control was 0,56%, 0,18% and 0,25%. The corresponding values of t were 2,7, 0,7 and 1,1, i.e., the difference between the experiment and control was statistically proven in only one series.

In the fourth series, some increase in relative liver weight was observed in the experiment, the difference between the experiment and the control (0,11%) was not, however verified statistically ($t \approx 0,6$).

The inhibition of liver growth caused by injecting a homogenate from tadpoles at earlier development stages than the recipients was also specific. There was no statistically proven decrease in the relative weight of the pancreas or anterior extremity in the experiment, as compared with the control, observed in any of the second group of experimental series. In two series, the relative weight of the pancreas in the control exceeded that in the experiment (0,028%, 0,021%), while, in the other two series, the relative weight of the pancreas was slightly greater in the experiment than in the control (differences of 0,021% and 0,036%, not statistically proven).

TABLE 2

Change in Relative Weight of Liver, Pancreas and Anterior Extremity of R. ridibunda Tadpoles Caused by Injection of Liver Homogenate from R. ridibunda Tadpoles at Stages 0-I.

Animal group	Recipient	Stage	Duration of experiment in days	Number of tadpoles	Relative weight of liver	Relative weight of pancreas	Relative weight of anterior extremity	t of Styudent's formula for relative wt. of liver in exp. and in control	P, according to Styudent's tables
Experiment	R. ridibunda	II	3	12	1.81	0.447	0.073	0.6	0.5485
Control				13	1.70	0.426	0.079		
Experiment	The same	II	4	12	2.23	0.505	0.119	2.7	0.0069
Control				10	2.79	0.533	0.143		
Experiment	" "	III	3	16	2.96	0.469	0.274	0.7	0.4839
Control				12	3.14	0 433	0.260		
Experiment	" "	III	6	11	1.74	0.376	0.108	1.1	0.2713
Control				7	1.96	0.397	0.121		

Therefore, the proposal that the tissue extracts received from donors younger than the recipients have an inhibiting effect cannot yet be definitely accepted. This possibility needs further verification on a larger number of animals.

It was interesting to note that, in two series of the first experimental group, liver tissue from R. temporaria tadpoles specifically stimulated liver growth in the R. ridibunda tadpoles. The difference between the experiment and the control was quite substantial ($t = 4,5, 3,8$).

Therefore, it can be assumed that interspecies stimulation is possible, i.e., stimulation of organic growth by proteins not specific to the given species. Apparently, the only necessary condition for the effective action of the extract is that organ-specific proteins be present in it.

Further research is needed to confirm this hypothesis.

SUMMARY

Two groups of experiments were conducted on Rana temporaria and Rana ridibunda tadpoles.

In the first group Rana temporaria and Rana ridibunda tadpoles of the I, II and III stages were injected intraperitoneally with a liver suspension of tadpoles (Rana temporaria of the V stage) in a 0,75% NaCl solution. In the second group, tadpoles (Rana ridibunda) of the II and III stages were injected with a liver suspension of tadpoles of the 0 and I stages of the same species.

In the first group the growth of the liver was specially stimulated by the homogenate, in the second group, a tendency to diminution of the relative weight of the liver was noted.

LITERATURE CITED

[1] G. D. Tumanishvili, K. M. Dzhandieri and I. K. Svanidze, Doklady Akad. Nauk SSSR, 1956, Vol. 106, No. 6, pp. 1107-1109.

[2] G. D. Tumanishvili, K. M. Dzhandieri and I. K. Svanidze, Doklady Akad. Nauk SSSR, 1956, Vol. 107, No. 1, pp. 182-184.

[3] G. Andres, Journ. Exper. Zool., 1955, Vol. 130, No. 2, pp. 221-249.

[4] I. D. Ebert, Proc. Natl. Acad. Sci. U.S., 1954, No. 5, Vol. 40, pp. 337-347.

[5] G. Levander, Nature, 1945, 155, pp. 148-149.

[6] F. A. McJukin and H. C. Breuhaus, Arch. Path., 1931, Vol. 12, pp. 900-908.

[7] H. Teir, Nord. Med., 1953, Bd. 50, N 28, S. 982-982.

[8] P. Weiss, Science, 1952, Vol. 115, No. 2992, pp. 487-488.

[9] P. Weiss and H. Wang, Anat. Rec., 1941, Vol. 79, No. 3, pp. 62-63.

CHANGE OF NUCLEIC ACID CONTENT IN STRUCTURAL COMPONENTS OF THE OVARIES OF WHITE RATS FED DIETS VARYING IN PROTEIN CONTENT

Z. A. Ryabinina

From the Laboratory of Growth and Development (Head of the Laboratory – Prof. L. D. Liozner), the Institute of Experimental Biology (Director – Prof. I. N. Maisky), Academy of Medical Sciences USSR, Moscow

(Received December 12, 1956. Presented by Prof. N. N. Zhukov-Verezhnikov, Active Member of the Academy of Medical Sciences, USSR)

In earlier published works [2], we discovered the structural and functional changes in ovaries which resulted from prolonged protein starvation followed by a well-balanced diet. The changes resulting from the starvation were expressed by growth retardation, delayed follicular differentiation and intensification of follicular atresia at the intermediate stages of follicular development, which led to a cessation of sexual development in immature rats and, in mature rats, to a cessation of the estrual cycle. In the ovaries of the starved rats, the primordial follicles were better preserved than the other structural components. After the animals had been transferred to a well-balanced diet, the structure and function of the ovaries was restored.

The purpose of this work was to study the changes in the ribonucleic and desoxyribonucleic acids in the structural components of ovaries in rats which had first undergone prolonged protein starvation and then been put on a balanced diet.

The studies were done on immature rats weighing 45-55 g and on sexually mature rats weighing 140-156 g. A total of 150 rats were used. The animals were kept on a synthetic diet, with a 19.5% protein content in the control and a 1.5% protein content in the experimental rats. The fat, vitamin and mineral content of both diets was the same. The immature rats were kept on the protein-deficient diet for a period of 23-64 days, the mature rats, for 45-135 days.

After long protein starvation, 57 rats (mature and immature) were put on the control diet. The immature rats were sacrificed on the 4th, 7th, 14th, and 17th days after the change of diet, and the mature rats, on the 14th, 25th and 50th days.

The animals were all sacrificed by decapitation; the mature rats were always killed during the diestrus period. The ovaries were fixed in Zenker-formol and by Gelli's method, and the material was imbedded in paraffin. Ribonucleic acid (RNA) was determined by Brashe's method; sections 6 microns thick were stained with methyl green-pyronine according to Unna-Pappenheim, and some preparations were first processed with ribonuclease and then stained in the same way. Desoxyribonucleic acid (DNA) was shown by Feigen's reaction, with the preparations first stained light green.

Examination of the ovarian sections from the control rats, mature and immature, showed that the oocyte of the primordial follicle contained a large, bladder-like nucleus, rich in DNA, which was distributed in granules around the nucleolus, the nuclear membrane and on the threads of the linin net. One, or occasionally two nucleoli, which stained well with pyronine, were usually distributed eccentrically in the nucleus; the oocyte cytoplasm contained a considerable amount of RNA.

In the follicular cells, DNA was distributed over the entire nucleus in the form of large granules. The

nuclei contained one roundnucleolus, rich in RNA. The cytoplasm of these cells contained much RNA (Fig. 1,a).

DNA distribution in the oocyte nucleus changed according to the degree of growth and maturity of the primordial follicles and their transformation into young follicles; there were many small as well as large granules of DNA which were centrally placed around the nucleole, or sometimes more to one side of it. The space nucleolus around the nuclear membrane was usually free of DNA.

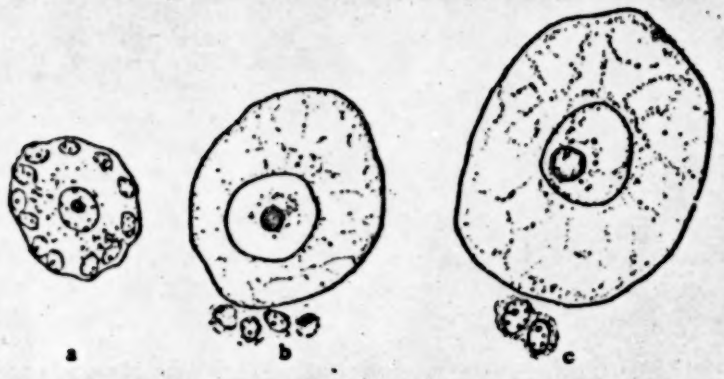

Fig. 1. Structural components of ovary in a control rat. Stained with methyl green-pyronine.
a) primordial follicle; b) oocyte of young growing follicle; c) oocyte of graafian vesicle.

The nucleolus was round, placed either in the center or to one side of the nucleus, and sometimes two nucleoli were observed; there was less RNA in the nucleolus than in the primordial follicles. There was either no RNA in the cytoplasm, or only traces of it around the nucleus and periphery of the oocyte.

The nuclei of the follicular cells in the young growing follicles were larger, rounder and richer in DNA, which was distributed throughout the nucleus in large granules. The cytoplasm of the follicular cells contained a considerable amount of RNA (Fig. 1,b).

The amount of DNA in the nucleus changed sharply during the process of growth and differentiation of the young follicle and its transformation into a graafian vesicle; the amount of RNA in the nucleolusdecreased, and there was no RNA in the cytoplasm.

We did not observe any material changes in DNA distribution in the nuclei of the follicular cells nor in the amount of RNA in the nucleolus and cytoplasm of these cells but the DNA granules did become larger (Fig. 1,c).

The interkinetic nuclei of the cells of the young corpora lutea contained a small quantity of DNA – small lumps and granules of DNA were distributed throughout the entire nucleus. The lutein cells nucleoli contained a large amount of RNA and stained intensively with pyronine. One could observe the nucleoli coming out into the cytoplasm of the lutein cells, a process which, when observed completely, began with the approach of the nucleolus to the nuclear membrane and ended with its complete escape into the cytoplasm.

The cytoplasm of the young corpora lutea cells contained a small amount of RNA, which was mainly distributed around the nucleus. Some of the young corpora lutea cells were mitotically dividing, and the nuclei of such cells were rich in DNA, but their cytoplasm contained less RNA than the cytoplasm of the cells whose nuclei were interkinetic.

In the regression stage of the corpus luteum, its cells became small, the amount of DNA in its nuclei

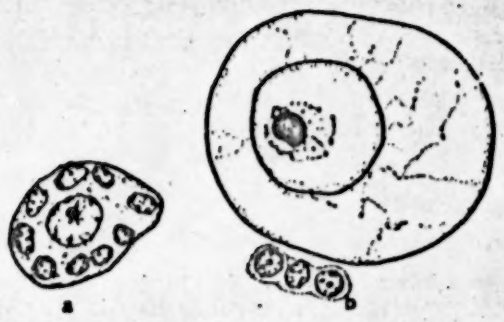

Fig. 2. Structural components of a rat's ovary after protein starvation. Stained with methyl green-pyronine. a) primordial follicle; b) oocyte of young follicle.

decreased, the nucleoli stained weakly with pyronine and RNA either completely disappeared from the cytoplasm or remained in a small amount.

Prolonged protein starvation causes a series of changes in the structural components of ovaries in mature and immature rats. These changes were of the same type, and we shall therefore give a general description of them below.

The DNA granules in the nucleus of the primordial follicle oocyte became coarser and were principally distributed around the nuclear membrane.

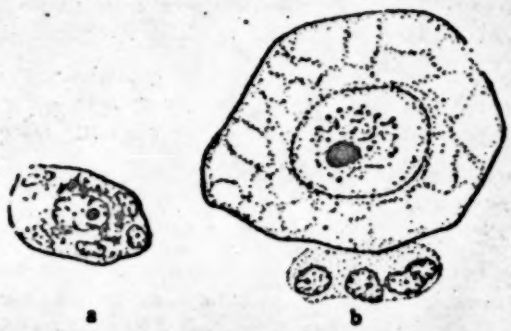

Fig. 3. Structural components of a rat's ovary during the restoration period. Stained with methyl green-pyronine. a) primordial follicle; b) young growing follicle.

The amount of RNA in the nucleolus and cytoplasm of the oocyte became somewhat smaller than the amount found in the primordial follicle oocyte from the control rat's ovary, but RNA did not, however, completely disappear.

In the nuclei of the follicular cells, DNA was distributed in coarser granules throughout the nucleus; the nucleolus and cytoplasm of the follicular cells contained less RNA and began to stain less intensively with pyronine (Fig. 2, a).

The following changes were observed in the oocytes of the young follicles: the DNA granules became larger and were mainly concentrated around the nucleolus; the amount of RNA in the nucleolus decreased; there was no RNA evident in the cytoplasm.

The DNA granules in the nuclei of the follicular cells became larger than the cells of normal ovaries. The cytoplasm and nucleoli of the follicular cells contained little RNA (Fig. 2, b).

In spite of the prolonged protein starvation, many follicular cells of the young follicles appeared in a state of karyokinetic division.

There were no undamaged mature graafian vesicles found in the ovaries of the starved rats.

During protein starvation, a series of changes also occurred in the corpora lutea of the mature rats. The amount of DNA granules in the nuclei of the lutein cells decreased, the nucleolus contained a small quantity of RNA and no RNA was found in the cytoplasm of the lutein cells.

All the corpora lutea of the starved rats were in the regression stage.

Consequently, protein starvation causes the quantity of RNA to decrease in the nucleoli and cytoplasm of all the ovarian structural components studied. One must note that the changes mentioned were most weakly expressed in the primordial follicles, which were better preserved than the other structural components in the ovaries of immature and mature rats.

After prolonged protein starvation, some of the animals were switched to a diet rich in protein.

The ovarian structural components of the immature rats changed considerably after 4 days of the balanced diet. The amount of RNA in the nucleoli and cytoplasm of the primordial follicle oocytes became greater than that in the primordial follicles of the ovaries in the starved rats.

The nuceli of the follicular cells of the primordial follicles retained the large, coarse granules of DNA, and the amount of RNA in the cytoplasm and nucleoli of these cells retumed to normal (Fig. 3, a).

In the young, maturing follicles, the amount of RNA in the nucleolus and cytoplasm of the oocyte increased.

The nuclei of the follicular cells still retained the coarse granules of DNA, and the amount of RNA in the nucleoli and cytoplasm of these cells increased (Fig. 3, b).

The maturing primordial follicles and the graafian vesicles in the ovaries of immature rats which had been put on a balanced diet after protein starvation (balanced diet for 14-17 days) were morphologically and cytologically the same as the corresponding structural components in the ovaries of the control animals.

The study of ovarian structure in mature animals put on a balanced diet for a period of 4-7 days following protein starvation showed a picture essentially the same as that found in the ovaries of the starved animals; the gonads were therefore studied at later dates after the restorative diet had begun (14th, 25th and 50th days).

After 14 days of the balanced diet, a series of changes was observed in the cytological structure of the ovarian structural components in mature, starved rats. For example the amount of RNA in the nucleolus and cytoplasm of the primordial follicle oocytes increased to the amount found in the control rats.

The amount of RNA also retumed to normal in the cells of the follicular epithelium.

As to DNA distribution in the nuclei of the oocytes and follicular epithelium of the young follicles, the ovaries of the experimental rats were the same as those of the control rats. The nucleoli contained a large amount of RNA.

When we examined ovarian sections from mature animals which had been put on a balanced diet after long protein starvation (duration of experiment: 25 days), we found that the ovarian structure and cytoplasm properties of these rats were the same as in the control animals.

Normal oogenesis was restored in the ovaries of the mature rats after 50 days of the balanced diet. Estrus

was observed in all of the animals 3-4 times during this period.

In the ovaries of rats fed a balanced diet for 25-50 days, the phenomenon of nucleolar escape from the nucleus was observed in the lutein cells; moreover, it was of a massive character.

The phenomenon of nucleolar escape which was observed in the corpus luteum cells could be considered as the manifestation of intense nucleic acids exchange between the nucleus and cytoplasm, which, in its turn, indicates the intensification of protein synthesis in the lutein cells.

The phenomenon of nucleolar escape was observed by Belousova [1] in the liver cells of rats fed a balanced diet after protein starvation.

As our studies have shown, long protein starvation causes the amount of DNA in the nuclei and the amount of RNA in the nucleoli and cytoplasm of the corpora lutea and of the follicles at various stages of maturity to decrease which indicates the decrease of nucleoprotein exchange in the ovarian structural components.

When a balanced diet is restores, so is the nucleoprotein exchange in the rats' ovaries, which is expressed by the increased amount of DNA and RNA in the cells of the structural components.

The cytological changes caused by protein starvation are less apparent in the primordial follicles, where the amount of DNA and RNA returns to the normal sooner than in the other structural components of the ovary.

SUMMARY

In sexually mature and immature rats kept for a long period of time on a protein-deficient diet (1.5% of protein) a fall of nucleoprotein metabolism in the structural components of the ovaries was observed. It was manifested by a decrease in the quantity of DNA in the nuclei and RNA in the cytoplasm and nucleoli of the primordial graafian follicles and corpus luteum. Cytologic changes were less manifested in primordial follicles.

When the rats were subsequently put back on a balanced diet (19.5% of protein) the quantity of DNA and RNA in the cells of structural components rose to normal, and the nucleoprotein metabolism in the ovaries was restored.

LITERATURE CITED

[1] A. K. Belousova, Voprosy Med. Khim., 1951, Vol. 3, p. 25.

[2] Z. A. Ryabinina, Doklady Akad. Nauk SSSR, 1952, Vol. 36, No. 4.

DETERMINATION OF BIOGENIC STIMULATORS BY THE YEAST NEPHELOMETRIC TEST

A. F. Sysoev and L. A. Martsinkevich

From the Academician V. P. Filatov Ukrainian Institute of Eye Diseases (Director – V. P. Filatov), Odessa

(Received March 18, 1956. Presented by Academician V. P. Filatov)

As V. P. Filatov [12] and other authors have established, an accumulation of biologically active substances (called biogenic stimulators) occurs in isolated tissues and in whole bodies during the action on the latter of unfavorable factors.

Biological tests are usually used to determine the accumulation of these biogenic stimulators, their chemical nature and action mechanism. The following are some of the tests proposed for this purpose: 1) acceleration of skin defect regeneration in animals [8, 14]; 2) acceleration of epithelial regeneration in isolated frog's eyes [5]; 3) acceleration of plant seed germination and development [2, 10]; 4) heightened enzymatic activity [1, 10]; 5) acceleration of yeast fermentative activity [13].

Some of these tests take a long time. For example, the test of accelerated skin defect regeneration, using a rabbit's ear, takes 30-40 days and needs a large number of experimental animals; the test on plants takes 10-15 days. Therefore, it is important that new tests, which would be simple, quick and exact, be discovered and developed.

Many authors [4 and others] have often mentioned the extraordinary sensitivity of yeast cells to various substances. With this in mind, V. P. Filatov, V. A. Biber and L. A. Adamanis [12] proposed that the fermentative activity of fresh bread yeast be used as a test to disclose biogenic stimulators. It was then discovered that this yeast often varies as to original fermentative activity, which distorts the results in experiments determining biogenic stimulators and does not permit comparison of results from different experiments. The method of L. I. Palladina and A. M. Gudina [6], which is based on stimulating dried bread yeast reproduction, has the same drawback.

To eliminate the biological heterogeneity of yeast cells, N. V. Yanyk and E. P. Karpova [14] proposed that a pure culture of yeast cells be used and their division be computed by counting the yeast cells in a chamber under a microscope. This method, however, has not been widely used since counting the cells in a chamber is a laborious, time-consuming method and is not free from the element of subjectivity.

This method was somewhat simplified by S. R. Muchnik and A. F. Shcherbina's modification of it. Computation of the cells under a microscope was replaced, in this modification by computation of the yeast colonies obtained by culturing the yeast in Petri dishes on a hard agar medium.

A. F. Sysoev [8] successfully used a method measuring yeast reproduction by an electrophotocolorimeter instead of by counting the yeast cells.

G. S. Dyachkov [3] indicated the possibility of determining the concentration of the yeast suspension by a nepheloimeter.

The purpose of this work was to find the optimal conditions for the use of the nephelometric method and to examine the possibility of converting it into a method allowing the activity of the tissue preparations used in different experiments to be compared, which is extremely important to the standardization of tissue preparations.

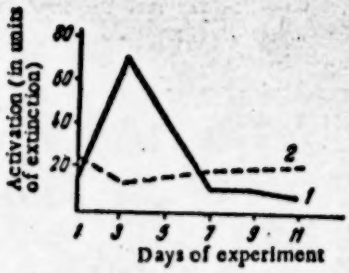

Fig. 1. Activation of yeast reproduction by extracts from preserved tissues compared with that effected by extracts from fresh tissues, depending on the age of the yeast culture (in units of extinction): 1) extract from preserved bull's skin; 2) extract from preserved aloe leaves.

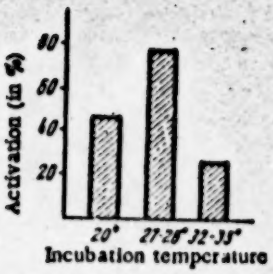

Fig. 2. Effect of yeast reproduction stimulation depending on incubation temperature.

The following questions were considered in our study: 1) the effect of the yeast culture's age; 2) the effect of the temperature in which the yeast was incubated; 3) the effect of the extract concentration on the degree of yeast reproduction; and 4) the frequency of similar results in experiments done at different times.

We believed that the age of the yeast culture was probably extremely important in this test, since it may be assumed that as the age of the yeast cell changes, so does its reaction to the influence of stimulating substances. We compared yeast culture reproduction in yeast of different ages in the same experiment and found that yeast aged 1-3 days reacts most actively to the stimulating substances of the preserved tissue extracts. With the addition of aloe extract to the yeast suspension, the age of the yeast did not seem to be so important, although the intensity of yeast reproduction decreased 2-3 times (Fig. 1) when an older yeast culture was used (7-11 days).

The influence of the incubation temperature on yeast reproduction stimulation was examined at temperatures of 20°, 27-28° and 33-34°. It was then established that the most optimal temperature was 27-28° (Fig. 2).

Data showing the influence of the extract dilution on the intensity of yeast reproduction is given in Table 1.

As the data in Table 1 show, yeast reproduction activation caused by adding an extract from preserved muscular tissues is greatest when the concentration of the extract is 1:5. When the extract is further diluted, the difference between the extracts from fresh tissues and those from preserved tissues is still apparent in dilutions of up to 1:50; extracts with greater dilutions have only a slight influence. As the aloe leaves extract was diluted, the stimulatory effect of the extract from the preserved leaves first increased and reached it strongest expression in a dilution of 1:50. The limit of sensitivity of the test of this extract must evidently be considered a 1:100 dilution.

Based on the data given above, we can assume that the most optimal conditions for the use of the yeast test are the following: age of yeast culture – 1-3 days (better to keep to some one of these days), incubation temperature – 27-28°, dilution of extract from animal tissues – from 1:5 to 1:50, from aloe leaves – 1:50 to 1:100 per wet weight of tissue.

EXPERIMENTAL METHODS

The experiments were done on a pure culture of Saccharomyces cerevisiae yeast, strain No. 47, using Rider's medium.

The method we developed is as follows: sterile test tubes (with cotton plugs) were filled, under sterile conditions, with 1 ml of the experimental extract in a corresponding dilution (usually 2 dilutions were tested – one from the optimal zone and another more concentrated). Then 4 ml of Rider's solution was poured into the test tubes (contents of Rider's solution, per 1 liter: 3 g ammonium sulfate, 1 g monohydrogen potassium

phosphate, 0,1 g dihydrogen potassium phosphate, 0,4 g calcium nitrate, 0,5 g sodium chloride, 0,7 g magnesium sulfate, 20 g saccharin), and 2 ml of a yeast suspension from a pure culture of Saccharomyces cerevisiae yeast with an extinction of 0,05 (optical density), according to the electrophotocolorimeter was added. We prepared the yeast suspension in the following manner: first a suspension was prepared from a 3-day old yeast culture on brewing wort. The pure yeast culture was maintained continuously by culturing two loops of liquid yeast culture, or one loop of the culture from the hard agar medium, in 5 ml of brewing wort. To obtain the yeast suspension, the pure yeast culture, in the 5 ml of wort, was poured into 100 ml of boiled tap water and shaken for 20 minutes by an apparatus for that purpose. Six ml were taken from this suspension and transferred to the next 100 ml of water. After this had been shaken for 10 minutes, the extinction of the resulting yeast suspension was determined; it had to measure 0,05 on the scale, and the layer of fluid in the vessel had to be 1 cm thick. If these measurements were not obtained, more water or more of the concentrated yeast suspension was added, depending on which was necessary.

TABLE 1

Activation of Yeast Reproduction Effected by Extracts from Preserved Tissues (in per cent of Reproduction Intensity Effected by Extracts from Fresh Tissues) with Different Dilutions of the Extracts.

Extracts	Dilution of extracts per wet weight of tissue							
	1 : 5	1 : 10	1 : 20	1 : 50	1 : 100	1 : 200	1: 500	1 : 1000
From bull's muscles .	150	140	134	130	104	108	109	108
From aloe leaves . .	123	132	159	160	146	143	—	106

EXPERIMENTAL RESULTS

We observed that the yeast remained cloudy for a day after acid fixation, and that the cloudiness sometimes recurred.

Biological activity was expressed figures of extinction (optical density) after taking the reading from the control test tubes and then translated per 1 mg of the dense residue of the extract.

This methods permits biological activity to be determined in many types of extracts simultaneously. The advantages this method possesses over other methods of determining biogenic stimulators is the speed and simplicity of its execution, the availability and economy of the materials needed and, finally and most important, the objectivity of its results and the possibility of comparing data obtained from different experiments.

The following example is a good example of this: in two experiments done at different times, the same extracts were tested, but with a different list of ciphers.

Water was poured into the control test tubes (there were 8-10 of these) instead of the extract. When the yeast suspension was poured into the test tubes, the flask containing the suspension was shaken constantly to prevent the yeast cells from settling. The test tubes were carefully shaken and placed in an incubator at a temperature of 27-28° for approximately 17-20 hours. After the 17 hour period in the incubator, the first two control test tubes were taken out and 1 ml of 50% by volume sulfuric acid was added to stop yeast growth; then the test tubes were shaken and the extinction was determined on an electrophotocolorimeter – it had to be 0,100 units on the scale. If the extinction had not yet reached this figure, the test tubes were left for another hour in the incubator, after which the extinction was again determined on the next two control test tubes, etc., until an extinction of 0,100 units was reached.

Measuring the concentration of the original yeast suspension and that of the control test tubes after incubation established a background for this test which was constant, thereby permitting the data obtained in differ-

ent experiments to be compared.*

After the indicated density of the yeast cell suspension was obtained in the control test tubes, all the test tubes were removed from the incubator, 1 ml of 50% sulfuric acid was then added, the test tubes were carefully shaken, poured into the electrophotocolorimeter container, and the extinction was measured. Water was poured into the control vessel.

TABLE 2

Results Obtained from Testing the Extracts

Extracts	Experiment I	Experiment II
From fresh aloe leaves	118	119
From preserved aloe leaves	123	129
From fresh placenta	123	119
From preserved placenta	135	130

The data in Table 2 shows the closeness of the results from both experiments and, therefore, the possibility of using the proposed test in order to standardize tissue preparations.

It could be proposed that the suggested yeast test method could also be widely used to determine other biologically active substances, vitamins, hormones, auxins, etc., which, added to a yeast suspension, would stimulate or inhibit yeast reproduction.

SUMMARY

Optimal condition for the A. F. Sysoev's yeast nephelometric test have been established. They are: age of yeasts 1-3 days, incubation temperature 27-28°; suspension of animal tissue 1 : 50 to 1 : 100 (live weight of tissue). The method thus modified may be used for standardization of tissue preparations.

LITERATURE CITED

[1] V. A. Biber and K. M. Magaziner, Doklady Akad. Nauk SSSR, 1951, Vol. 76, pp. 609-616.

[2] A. V. Blagoveshchensky and A. Yu. Kologrivova, Doklady Akad. Nauk SSSR, 1945, Vol. 48, No. 8, pp. 599-602. 599-602.

[3] G. S. Dyachkov, Mikrobiologiya, 1941, Vol. 10, No. 6, pp. 771-776.

[4] S. Ya. Zalkind, Uspekhi Sovremennoi Biol., 1952, Vol. 34, No. 3 (6), pp. 473-477.

[5] S. R. Muchnik, Studies of the Western Ukrainian Experimental Institute of Eye Diseases,* 1955, Vol. 3, Vol. 3, pp. 303-309.

[6] L. L. Palladina and A. M. Gudina, Vrach. Delo, 1950, No. 3, pp. 227-238.

[7] V. V. Skorodinskaya, Studies of the Western Ukrainian Experimental Institute of Eye Diseases, 1949, Vol. I, pp. 114-120.

[8] A. F. Sysoev, in the book: Tissue Therapy,** Kiev, 1953, pp. 29-44.

[9] A. F. Sysoev and V. V. Skorodinskaya, Vestn. Oftalmol., 1951, No. 4, pp. 24-32.

*Since it is very difficult to ascertain if the readings of the control test tubes are exactly 0.100 on the scale, one may equalize the data by rounding it off to 0.100. The permissible deviations are between 0.090 and 0.120.

**In Russian.

[10] A. F. Syaev and S. P. Skripchenko, Studies of the Western Ukrainian Experimental Institute of Eye Diseases, 1952, Vol. 2, pp. 30-34.

[11] V. P. Filatov, Izv. Akad. Nauk SSSR, Ser. Biol., 1951, No. 6, pp. 23-59.

[12] V. P. Filatov, V. A. Biber and L. I. Adamants, Studies of the Western Ukrainian Experimental Institute of Eye Diseases, 1949, Vol. 1, pp. 106-113.

[13] V. P. Filatov and A. F. Syaev, Vrach. Delo, 1950, No. 3, pp. 197-204.

[14] N. V. Yanyk and E. P. Karpova, Studies of the V. P. Filatov Western Ukrainian Experimental Institute of Eye Diseases, 1954, Vol. 3, pp. 66-73.

MORPHOLOGY AND PATHOMORPHOLOGY

INTERNEURONAL SYNAPTIC CONTACTS IN THE PERIPHERAL SECTION OF THE VEGETATIVE NERVOUS SYSTEM

V. I. Pilipenko

From the Neurohistologic Laboratory (Chief – E. K. Plechkova) of the Institute of Normal and Pathologic Physiology (Director – Active Member of the Acad. Med. Sci. USSR Prof. V. N. Chernigovsky) Acad. Med. Sci. USSR, Moscow

(Received August 28, 1956. Presented by Active Member Acad. Med. Sci. USSR Prof. V. N. Chernigovsky)

Clinical observations as well as experimental studies have proven that internal organs preserve their functional capacities, up to a certain extent, even when there is complete rupture of communications with the central nervous system, this giving the organism a greater ability to survive. It is for this reason that there is a special significance in the study of the construction of the peripheral nerve ganglia, the clarification of their morphology and the connections existing between the intramural and the extramural ganglia. As is known, A. S. Dogiel [12] believed that the peripheral ganglia possess sensory neurons (Type II cells) which have synapses with motor neurons (Type I cells), this forming a reflex arc.

However, after the studies by John Langley [17], it was generally accepted that the peripheral neurons are effector only and that in the ganglia there is merely a transmission of the nerve impulse from the pre- to the postganglionic fibers.

There are many studies indicating that A. S. Dogiel was right in ascribing sensory capacities to cells of Type II and that there are indeed synaptic ties among peripheral neurons [1,3,6,7,8,9,11,16].

We were able to demonstrate along with I. F. Ivanov [1] that severing mesenteric nerves leads to degeneration of the proximal or central portions of the nerve and that this degeneration can be followed into the ganglia of the solar plexus. Under such conditions there is an alteration in the synaptic apparatus of the nerve cells composing the solar plexus [8].

The aim of the present study is to analyze the interneuronal connections existing in the solar plexus when its cells lose all ties with the central nervous system.

EXPERIMENTAL METHODS

We developed a procedure involving the total extirpation of the spinal cord in cats, followed by a series of measures permitting these animals to live long enough to show the transformations of the preganglionic fibers which occur at the synapses of the peripheral ganglia.

When the required care was given the animals would survive for quite some time, and so proved to be a convenient animal in which to study the many problems of the peripheral nervous sytem, its morphology and physiology, the activity of internal organs, the regulation of peripheral circulation and so on.

The operation was performed under ether-chloroform narcosis.

In two places: at the level of thoracic 5-7 segment and, then, (after severing cord) at the sacral segment we opened paths to the spinal cord, first making a long skin incision, separating muscle attachments from the bodies and processes of the vertebra and finally opening the spinal canal by means of Luer forceps.

To perform the operation successfully, especially considering its immediate postoperative condition, local anesthesia is required. It is especially important to remove sensation while the spinal cord is being manipulated. It is for this reason that, having made the small aperture into the thoracic portion of the spinal canal, we would immediately introduce through it 0,2 cc of 1% novocaine. After a lapse of some time novocaine would also be introduced into the subdural space. Still later small quantities of novocaine were put very gradually into the various levels of the thickness of the spinal cord itself. It can be seen that the use of these procedures resulted in all manipulations of the cord, including the sectioning, being done under complete local anesthesia, independent of the general narcosis.

At the very end of the operation we introduced novocaine into the entire thickness of the central portion of the severed cord creating a sort of a local depot.

After severing the cord, the ends of the isolated portions were ligated. The dura matter had to be included in the ligatures, this facilitating the removal of the spinal cord from the vertebral canal. For the same reason the maximum number of spinal roots that could be reached were severed.

The spinal cord was pushed out by using a metal rod having the curve of the spinal canal. Simultaneously, there was exerted a pull on the ligature holding the caudal end of the cord.

The portions of the spinal cord remaining within the spinal canal - were destroyed using as a cautery the heated end of a metal rod, this also helping control bleeding. After satisfactory hemostasis, the muscles and then the skin were sewn in layers. The animal's greatest postoperative dangers to life are excessive hemorrhage, , disturbance of heat regulation and the rather frequent severe upset of cardiac rhythm. For this reason we always gave immediately after operation, up to 20 cc of physiological saline subcutaneously (amount depending on weight of animal), camphor also being used when needed.

For the first postoperative day artificial heat would be given, the body temperature being measured at regular intervals thus allowing normal body temperature to be maintained.

For the first few days animals would refuse food and water, as a usual rule. Feedings were given by stomach tube milk and beef broth being used.

The system of postoperative care included periodic emptying of the bladder, pressure being used, and removal of feces from the rectum. With the aim of either preventing or minimizing trophic sores, the position of the animal would be altered at frequent intervals while, later, the posterior portion of the body as well as the rear limbs would receive frequent massages.

Altogether 8 cats were operated. One perished on the 4th day as a result of inspiration pneumonia. The others were sacrificed between the 12th and 21st days postoperatively. In the last operation a second operation was performed on the 15th day following removal of the spinal cord: sub-diaphragmatic vagotomy and section of the splanchnic nerve with the sympathetic chains. The aim of this second operation was to eliminate the possibility that vagal and sympathetic fibers originating from the first thoracic segments of the spinal cord would form synaptic apparati in the solar plexus.

The solar plexus was stained with silver using the Campos procedure, with the method of Gross-Bielschowsky using the B. I. Lavrentiev modification and with the Cajal pyridine procedure, the tissues being placed in celloidin.

EXPERIMENTAL RESULTS

Study of the preparations made showed that exclusion of the synaptic apparatus and the preganglionic nerve fibers of central nervous sytem origin greatly simplified and clarified the analysis of the structural relationships existing in the peripheral nerve ganglia.

Upon many nerve cells of the solar plexus there was demonstrated an elaborate synaptic apparatus, this being represented by a pericellular network of the very finest nerve fibers coming into the most intimate contact with the cell bodies themselves and their dendrites. It cannot be doubted that at least a portion of these fibers represent the axons coming from sensory neurons (Type II Dogiel) lying within the walls of the digestive tract. It is precisely these synapses that degenerate when the mesenteric nerves are severed, this being observed by us in our previous experiments. This is proven also, partially, by the observation that the altered synapses of the present experiments are seen most often and in the largest number on the periphery of the nerve ganglia.

Among the synapses of the solar plexus which remain intact after the removal of the spinal cord are axons from the cells of the Type II Dogiel, these forming an organic part of the solar plexus itself. Here can be seen also other forms of interneuronal communication, generally grouped under the term "horizontal connections" [2], these being recognized by the manner in which the dendrites or axons of one cell make contact with the dendrites and cell bodies of other cells, the anastomoses from cell to cell and so on.

The pericellular apparatus of the various cells composing the solar plexus attains unequal degrees of development. Some cells are thickly covered by a net of the finest nerve fibers which approach the cell from all sides (Fig. 1). Other cells are covered by only one or two such fibers (Fig. 2). Finally, in the third type, there is no pericellular apparatus that can be seen at all.

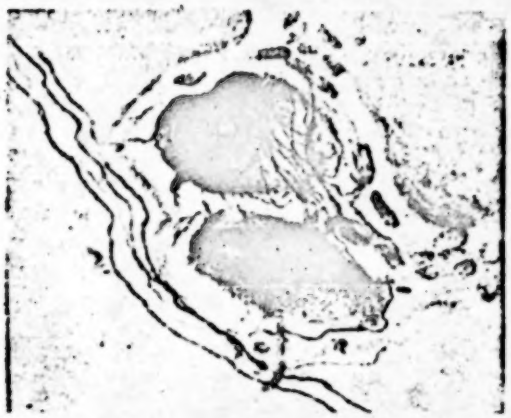

Fig. 1. The pericellular apparatus of a nerve cell from the solar plexus 19 days after extirpation of the spinal cord.

Only in exceptionally rare instances do these fine nerve fibers terminate as slight thickenings making contact with the bodies and extensions of the nerve cells. Somewhat more often, there can be seen on these uniformly thin, fine nerve fibers of the pericellular apparatus single, tiny, varicose-like thickenings which also make contact with the bodies of the nerve cells (Fig. 3).

It is of interest to note that the nerve fibrils from a nerve fiber may form part of the pericellular network around one cell body and then extend to the pericellular apparatus around adjoining nerve cell bodies. This makes possible the transmission of a nerve impulse from a single axon of a Type II Dogiel cell to several effector neurons just as is the case for preganglionic fibers of central origin ([Langley, 17], [S. I. Lavrentiev, 6], [Kirsche, 14], and others).

When the synaptic apparatus of the solar plexus is studied in the normal animal and then the same apparatus is compared with what remains of it after the spinal cord has been extirpated, substantial differences are uncovered between the synapses formed by axons of cell Type II Dogiel II and the synapses formed by preganglionic cell fibers of central origin. The synapses formed by the dendrites of peripheral sensory neurons form the finest of pericellular fibrils enlacing the cell bodies and only rarely do they terminate in button-like thickenings. In our experiments with sectioning the mesenteric nerves [8], it is precisely these synapses that became altered.

The synapses described in the literature [4, 14, 15], forming the typical loops or rings making contact with the cell bodies and their extensions similar in their pattern to what is observed in the central nervous system, belong to the preganglionic fibers of central origin. Kirsche [15] in one of his studies classifies the morphologic and physiologic peculiarities of the various synapses as those with a "large" or "small area of transmission."

The presentation of the data given above when added to that previously advanced testifies to the fact that the interneuronal connections within the solar plexus as well as in other peripheral connections within the solar

Fig. 2. Pericellular nerve fibers in the solar plexus 21 days after extirpation of the spinal cord and 6 days following sub-diaphragmatic vagotomy and sectioning of the splanchnic nerve.

Fig. 3. Terminal thickenings and thickenings along the course of pericellular fibrils (synaptic buttons) 21 days after extirpation of the spinal cord and 6 days after sub-diaphragmatic vagotomy and sectioning of the splanchnic nerve.

plexus as well as in other peripheral ganglia are considerably more complex than is assumed by the hitherto generally accepted, well-known Langley schematic representation.

The ganglia of the peripheral nervous system must be thought of, first of all, as peripheral reflex centers which play a most significant role in the regulation of the activities of the internal organs.

SUMMARY

A method of complete extirpation of the spinal cord in cats and postoperative care of the animals has been elaborated. A study of slides of the solar plexus from animals killed on the 12-21 day after extirpations con-

firmed the presence of interneuronal synaptic contacts in the peripheral section of the vegetative nervous system. Interneuronal contacts in the solar plexus are more complex than was previously thought due to the Langley schematic representation.

Ganglia of the peripheral nervous system should be regarded first of all as peripheral reflex centers which are very important to the regulation of the activities of the internal organs.

LITERATURE CITED

[1] L. F. Ivanov, Transactions of the Tatar Scientific Investigative Institute of Theoretical and Clinical Medicine,* Kazan, 1937, fourth ed., pp. 262-357.

[2] L. F. Ivanov, Transactions of Fifth All-Union Congress of Anatomists, Histologists and Embryologists,* Leningrad, 1951, pp. 307-309.

[3] T. S. Ivanova, Doklady Akad. Nauk, SSSR, 1952, Vol. 85, No. 4, pp. 901-904.

[4] G. A. Koblov, The Morphology of the Cells of the Solar Plexus (Histological Studies),* (1952).

[5] B. L. Lavrentiev, Morphology of the Autonomic Nervous System,* Moscow–Leningrad (1939).

[6] A. A. Miloklin, Problems in the Morphology of the Receptors of the Internal Organs and the Cardio-Vascular System,* Moscow–Leningrad, 1953, pp. 77-79.

[7] A. V. Nemilov, Transactions of the St. Petersburg Society of Naturalists * St. Petersburg, 1902, Vol. 32, No. 2, pp. 89-96.

[8] V. L. Pilipenko, Proceedings of the Scientific Congress on the Problems of the Physiology and Pathology of Digestion,* Kiev, 1954, pp. 129-131.

[9] V. L. Pilipenko, Byull. Eksptl. Biol. i Med., 1956, Vol. 41, No. 5, pp. 70-74.

[10] N. F. Popov, Nevropatol. Psikhiat. i Psikhogig., 1932, Vol. 1, No. 3-4, pp. 97-103.

[11] L. A. Chervova, Doklady Akad. Nauk SSSR, 1955, 103, No. 3, pp. 321-324.

[12] A. S. Dogiel, Arch. mikr. Anat., 904, Bd. 64, S. 173-188.

[13] F. Golz and A. Frensberg, Arch. A. ges. Physiol., 1874, Bd. 9, S. 174-197.

[14] W. Kirsche, Ztschr. f. mikr. anat. Forsc., 1954, Bd. 60, H. 3, S. 399-466.

[15] W. Kirsche, Ztschr. f. mikr. anat. Forsch., 1955, Bd. 6, S. 541-548; 624-640.

[16] A. Kuntz, J. Comp. Neurol., 1940, Vol. 72, pp. 371-382.

[17] L. Langley, J. auton. nervous system (1903).

* In Russian.

SOME FORMS OF INTERNEURONAL CONTACTS IN VEGETATIVE GANGLIA

I. D. Lev

From the Faculty of Normal Anatomy (Chairman – Corresponding Member of the Acad. Med. Sci. USSR Prof. B. A. Dolgo-Saburov), S. M. Kirov Military Medical Academy

(Received May 5, 1956. Presented by Active Member Acad. Med. Sci. USSR Prof. V. N. Chernigovsky)

The nerve cells of the vegetative ganglia are in morphological and physiological contact with the organs they innervate and with the nerve centers of the spinal cord and brain. These latter, called interneuronal, are represented by the pericellular net formed by the endings of the preganglionic fibers as they penetrate to the body of the nerve cell extending under its capsule and making contact as rings and buttons with the body and dendrites. There has been established the fact that a perifibrillar substance exists, surrounding the neurofibrillar frame of the synaptic ending of the preganglionic fiber [7, 10, 13].

In the studies made of the relations between the endings of the preganglionic fibers and the protoplasm of the bodies of the nerve cells within the vegetative ganglia, so far, but little attention has been devoted to the especially intimate contacts these have. It is assumed that at this very point [11] the impulse is transmitted across its special membrane using the biochemical physiologic meaning of that word [20]. One should not overlook mentioning the possible role played by the satellite cells of the nerve capsule in the transmission of the impulse from neuron to neuron by, for example, mediators of the acetylcholine type [16, 19]. These cell satellites, being elements of the peripheral glia, form along with the neurons and nutrient vessels a single morpho-physiological complex.

Further study of the vegetative ganglia has shown that they have not only efferent but also afferent neurons and so must be considered as containing a mixture of elements.

V. M. Bechterev [2] wrote that, "essentially, the ganglionic or sympathetic nervous system is really an off-shoot of the central nervous system and just as this latter consists of individual nerve elements or units conducting impulses centrally and peripherally so also the sympathetic system consists of a ganglionic chain composed of varying nerve elements touching and intercommunicating with each other, effector as well as sensory units being present these latter to transmit to the brain impulses from our inner organs." At almost the same time, A. S. Dogiel [17] divided the nerve cells of the vegetative system into three types and pointed to the cells of the second type as being sensory, the intrinsic receptors of the autonomic system. Soon afterwards A. S. Dogiel [18] and S. E. Mikhailov [14] discovered the presence in autonomic ganglia of sensory endings disposed in the stroma between the nerve cells. These receptors have been lately extensively examined by N. G. Kolosov [10] and his students and this group has described various intra- and extracapsular endings in the solar plexus, the bordering sympathetic chain, in ganglia around the larynx, bronchi, nodes of the digestive tract, mesentery and so on.

Inadequate knowledge of the stroma of the vegetative ganglia, particularly of its blood vessels, has so far made rather difficult the precise physiological evaluation of the receptors just described. It must be assumed that some of these receptors arise from the walls of the blood vessels [3, 4, 5], others are related to the stroma, neurons and the glia surrounding them [9, 10].

In addition, lately N. G. Kolosov [9, 10], T. A. Batireva [1], G. A. Koblov [8], Yu. I. Slepkov [15] and

others have demonstrated within the vegetative ganglia "afferent, pericapsular elements originating largely from coarse, myelinated fibers which, approaching the neurons, lose their sheath and fragment into several fine, nonmedullated fibers which terminate on the surface of the capsule of the nerve cell as rings or buttons" [10]. As can be noted, the principal morphologic differentiation from the already known pericellular apparatuses lies in the disposition of these fibers exclusively outside the capsule of the nerve cell. It is understood that the afferent character of this type of pericapsular apparatus can be finally proven only by using the method of sectioning the sensory nerve fibers and then observing the consequent degeneration. In the laboratory of N. G. Kolosov this has been done.

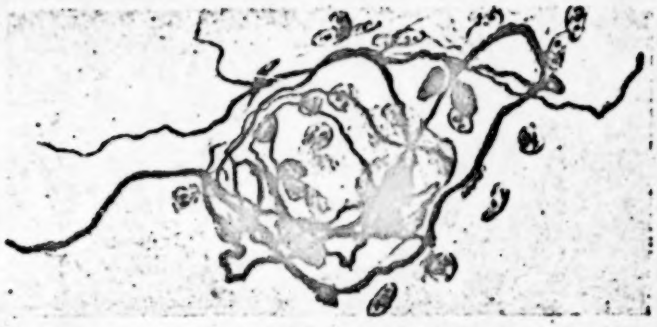

Fig. 1. Pericapsular ending formed by the terminal branchings of a coarse, myelinated fiber. Bielschowsky-Gross. Eye-piece x 10, obj. x 40.

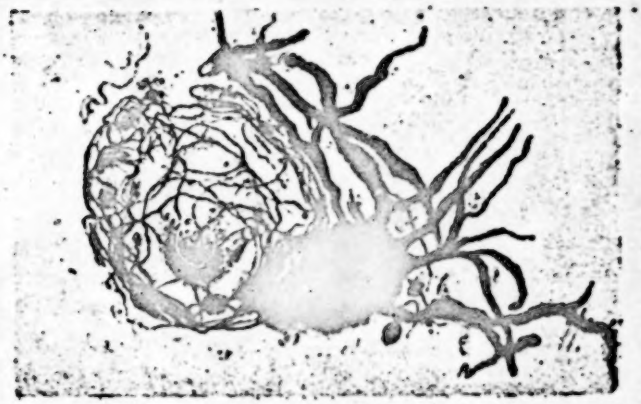

Fig. 2. Pericapsular network formed by fine nerve fibers. Impregnated after Bielschowsky-Gross. Eye-piece x 10, obj. x 40.

The next step is to accumulate data which will allow us to establish that the pericapsular apparatus is as much a structural part of the neuron as the pericellular apparatus.

In studying the nerve net of the human kidney, along with the pericellular apparatus, there were uncovered many structures having a close relation to the capsule of the nerve cells. The simplest of them are composed of a single or several nerve fibers forming a few branchings around the capsule (Fig. 1). More complex is

the structure represented by the heavy mesh of fine nerve fibers enveloping the capsule of the nerve cell in the manner of a cocoon (Fig. 2). Quite often it can be observed how one or several fibers, before entering into this mesh, wind spirally around a dendrite and only then continue over the outer surface of the capsule (Fig. 3). At the same time, it should be noted that between the capsule and the mesh covering it and, the body of the nerve cell, there is seen a distinct space into which the above-mentioned fibers do not penetrate and, hence, do not form pericellular apparatuses (Fig. 4).

Fig. 3. Pericapsular mesh formed by fine nerve fibers. One fiber winds in a spiral around a dendrite. Impregnation after Bielschowsky-Gross. Eye-piece x 10, obj. x 40.

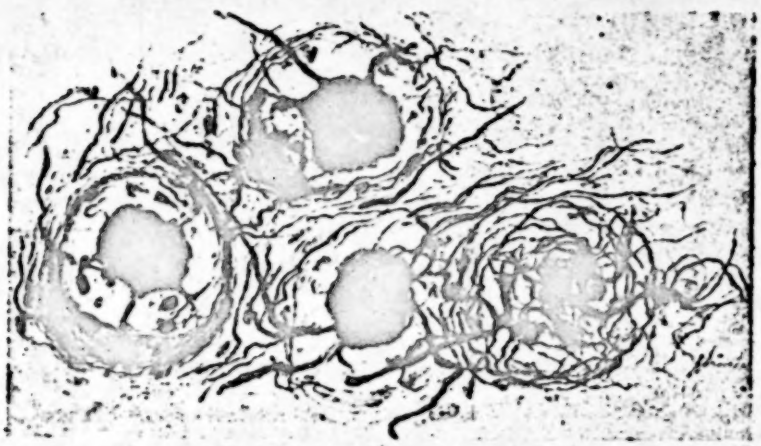

Fig. 4. Group of nerve cells having pericapsular networks. Fibers can be seen which wind spirally around dendrites. Impregnation after Bielschowsky-Gross. Eye-piece x 10, obj. x 40.

All this permits us to consider these structures as being different from the known types of interneuronal contacts forming pericellular apparatuses and so we may point to the similarity in our microphotographs to those presented by N. G. Kolosov [9,10] and others as "special afferent devices."

Quite obviously, so far it is difficult to assign a definite meaning to these pericapsular apparatuses in the autonomic ganglia. We might recall the words of B. I. Lavrentiev [12] who states, "each time that an impulse from a preganglionic fiber transfers to the neuron of the peripheral ganglion, the event is signalled to the central nervous system. In other words, the synaptic transmission in the ganglia of the vegetative system is under constant control by the central nervous system." Other theories, of course, might be advanced but it is far better to direct our attention to morphological and physiological experiments so devised as to be definitive in elucidating the problem of the peculiarities of the inter neuronal contacts in vegetative ganglia.

SUMMARY

Neural fibers forming pericapsular plexuses around the human renal kidney are depicted on the microphotographs shown. A clear space between the cell body and this plexus has been demonstrated. It is assumed that there is no pericellular apparatus and that the pericapsular apparatus demonstrated is, most probably, of an afferent character.

LITERATURE CITED

[1] T. A. Batkerva, Problems in the Morphology of the Receptors of the Internal Organs and the Cardio-Vascular System," Moscow–Leningrad, 1953, pp. 148-165.

[2] V. M. Bekhterev, Conduction Tracts of the Brain and Spinal Cord, Instructions to the Study of the Internal Pathways of the Brain."

[3] B. A. Dolgo-Saburov, cited by G. V. Orlovsky. Arkh. Patol., 1954, No. 2, pp. 72-74.

[4] B. A. Dolgo-Saburov, Zhur. Vysshei Nerv. Deyatel., 1954, Vol. 4, No. 6, pp. 903-908.

[5] B. A. Dolgo-Saburov, Doklady Akad. Nauk SSSR, 1955, Vol. 103, No. 3, pp. 521-524.

[6] Yu. M. Zhabotinsky, Normal and Pathological Morphology of the Vegetative Ganglia," Moscow (1953).

[7] G. A. Koblov, Arkh. Anta. Gistol. i Embriol., 1952, Vol. 29, No. 3, pp. 31-36.

[8] G. A. Koblov, Problems of Morphology, " Moscow, 1953, 2nd edition, pp. 96-109.

[9] N. G. Kolosov, Problems in the Morphology of the Receptors of the Internal Organs and the Cardio-Vascular System," Moscow–Leningrad, 1953, pp. 241-260.

[10] N. G. Kolosov, Innervation of Internal Organs and the Cardiovascular System, Moscow–Leningrad, 1954, p. 212.

[11] B. I. Lavrentiev, Morphology of the Autonomic Nervous System," Moscow, 1939, pp. 5-77.

[12] B. I. Lavrentiev, Morphology of the Sensory Innervation of the Internal Organs" Moscow, 1948, pp. 5-21.

[13] A. A. Maximov, Foundations of Histology" Leningrad, 1925, Part 2, Tissue Studies, pp. 295-296.

[14] S. E. Mikhailov, Vestnik Nevrol., 1909, 16, No. 2, pp. 320-335.

[15] Yu. I. Slepkov, Problems in the Morphology of the Receptors of Internal Organs and the Cardiovascular System, Moscow–Leningrad, 1953, pp. 213-214.

[16] F. De Castro, Arch. Internat. Physiol., 1951, Vol. 59, F. 4, pp. 479-525.

[17] A. Dogiel, Anat. Anz., 1897, Bd. 11, S. pp. 679-687.

[18] A. Dogiel, Der Bau der Spinalganglien des Menschen und der Säugetiere, Jena, (1908).

[19] V. Jabonero, P. Comez Bosque, F. Bordallo and Casas Perez, J. Der. anatomische Aufbau des peripheren neuro-vegetativen System. Neubearbeitung der ersten spanischen Auflage, Wein, 1953.

[20] W. Kinche, Ztschr f. mikroskop-anat. Forsch., 1954, Bd. 60, H. 3, S. 399-466.

" In Russian.

THE LYMPHATIC APPARATUS OF THE HUMAN TESTICULAR TUNICA PROPRIA IN RELATION TO ITS RESORPTIVE FUNCTION

E. B. Khaisman

From the Institute of Normal and Pathological Physiology (Director – Active Member Acad. Med. Sci. USSR Prof. V. N. Chernigovsky), Acad. Med. Sci. USSR, Moscow

(Received October 11, 1956. Presented by Active Member Acad. Med. Sci. USSR Prof. V. N. Chernigovsky)

The data relating to the lymphatic system of the tunica propria of the human testicle and the role played by it in the resorption of fluids and weighed suspensions, is quite scanty. There is only a single study by L. Allen [2]. In model experiments with human cadavera he demonstrated that injection of India ink into the cavity of the serous membrane of the tunica followed by light massage of the scrotum would, within a few minutes, result in entrance of this suspension into the lymphatic capillaries of the parietal layer of this covering. This author is of the opinion that lymphatic channels of the parietal layer play a major role in the absorption of fluids and suspensions present in the serous coverings of the testicle.

The work done by Allen deserves without a doubt the closest study even if many important problems having a direct bearing on the question in hand have remained either barely touched or altogether overlooked. It does not appear that he examined adequately the structure and especially the intra-serosal architecture of the lymphatic vessels lying within the serosal coverings of the testicle. There is no clarity in the mechanism leading to resorption of liquids and suspensions from the tunica into lymphatic channels. Finally, there has to be an experimental approach to the question of resorption by the tunica of hemorrhages.

All these enumerated problems furnished the object of the present investigation.

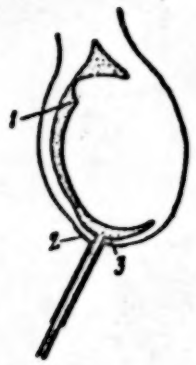

Fig. 1. Schematic representation of the cannulation of the serosal cavity around the testicle.

EXPERIMENTAL METHODS

In order to investigate the resorptive powers of the lymphatic vessels in the coverings of the human testicle, we employed cadavera of males, mostly of middle age, who had died from various types of trauma. In one experimental series we injected into the serosal cavity a suspension of India ink or Berlin blue (14 experiments), in another series – nucleated erythrocytes from hen blood which had been previously centrifuged repeatedly to separate the plasma and then weighed while in physiological saline suspension (10 experiments).

At the level of the lower pole of the testicle we opened all scrotal layers until the tunica propria was reached. The length of incision was 1-1.5 cm. In the wall of the tunica propria there would then be made a small opening (not over 0.5 cm), this leading directly into the cavity (Fig. 1, 1). Into this opening there was

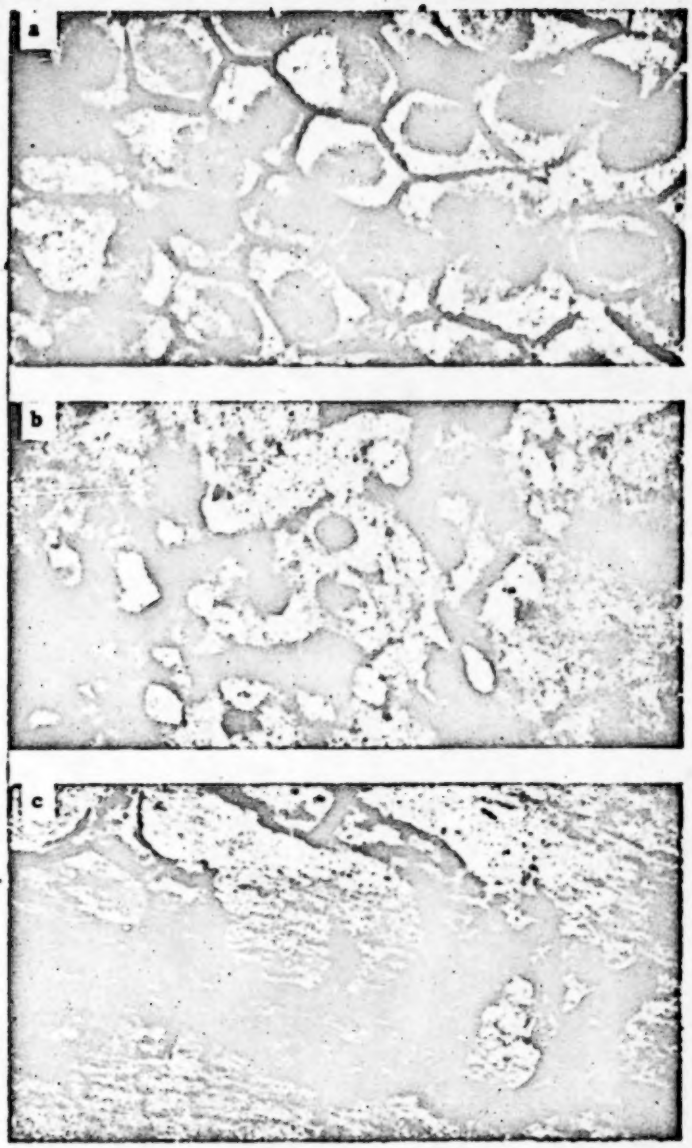

Fig. 2. Parietal layer of the tunica propria of human testicle. Into the space there had been injected 0,1% suspension of erlin blue (length experiment 5 minutes.

a) adsorption of dye particles at edges of mesothelial cells (nuclei stained with Ehrlich hematoxylin); immersion; b) window in basal membrane and nuclei of the lymphatic capillary endothelium; high power; c) the injected Berlin blue within lymphatic vessels of the deep net.

be introduced a glass cannula having a spherical rim 3 at the end. On this cannula was superposed cannula 2 having the same shape. In this situation the first cannula was fixed firmly to the wall of the cavity and the edges of the opening in the tunica were compressed between the rims of the two cannulas thus excluding the possibility that particles could enter the lymphatics through the incised tissues.

The inner cannula was then connected with a syringe or rubber bulb and the inside of the cavity was then rinsed out with physiological saline (to avoid clumping of suspensions) and only then would the indicator be carefully introduced until the level of the liquid in the cannula reached a predetermined level. Usually, not over 3 cc of suspension was injected.

Then the corresponding side of the scrotum was massaged, the tissues being alternately compressed and distended rhythmically by the fingers – all this varying the volume of the liquid that had been injected within the cavity of the coverings of the testicle. In this manner we attempted to duplicate the dynamics seen in life when the smooth musculature of the scrotum and the tunica of the testicle undergo rhythmic contractions [2, 5].

The experiments lasted from 2 to 30 minutes. At the end the cavity of the tunica propria of the testicle was fully opened and gently rinsed out with physiological saline, this removing the superficial particles of the indicator.

After fixing the tissues in 10% neutral formalin, longitudinal preparations were made from the entire visceral and parietal layers of the tunica propria. Depending on the aim of the microscopic analysis, the preparations were stained further with various reagents (iron trioxyhematin of Hansen, Ehrlich hematoxylin, aniline blue, fuchsin, etc.).

Fig. 3. Parietal layer of the human testicular tunica propria. Into the cavity nucleated hen red blood corpuscles were introduced (duration of experiment was 10 minutes). Erythrocytes can be seen within the lumen of the lymphatic capillary (Stained after Lepen and with Ehrlich hematoxylin). Immersion.

EXPERIMENTAL RESULTS

Even in experiments lasting 3-5 minutes, introduction of India ink or Berlin blue into the cavity of the tunica propria of the testicle would, as a rule, lead to intensive resorption of these indicators by the surface of the parietal layer while the visceral layer absorbed but traces of the dye. When the parietal layer was carefully scrutinized with the naked eye, it was possible to see that the lymphatic channels were becoming